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An investigation into the causes and
consequences of variability in community
structure in a large freshwater loch

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Degree of Doctor of Philosophy

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Biology

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Abstract

In order to explore the causes and consequences of variability in community structure in Loch Lomond, submerged macrophyte biomass values were assessed. The introduction of non-native species and changing nutrient levels are identified as threats to the macrophyte communities of the loch. Loch Lomond is diverse in habitat and this is evident in fish and invertebrate community structure. For a single fish species, there was evidence of between-site variability in a number of aspects of its ecology at relatively small spatial scales making Loch Lomond an ideal location for the investigation of the consequences of variability in community structure. An exploration was also made into a novel technique for the estimation of trophic niche width by use of the variance in stable nitrogen isotope signatures ($\delta^{15}\text{N}$) which lead to the conclusion that there are significant potential strengths in using $\delta^{15}\text{N}$ as a measure of trophic niche width. The $\delta^{15}\text{N}$ technique was used to explore a number of hypotheses related to the effect of community structure on trophic niche width, leading to the conclusion that the physical factors of a study site were more important than community structure in controlling trophic niche width of ruffe in Loch Lomond. Finally, the consequences for individuals of modified trophic niche width were investigated and lead to the conclusion that in Loch Lomond there was no clear relationship between trophic niche width and individual fitness.

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Chapter 1. Introduction

Much of the theory of ecology is built upon the ecology of populations. By examining how populations are limited by factors such as food and competition, we can begin to determine what controls the abundance of species in nature. Knowing what factors affect populations can help us combat species extinctions, lessen species endangerment, and maximise sustainable yields in fisheries and forests. Following on from this, community ecology focuses on why certain areas have high numbers of species compared with other areas that have low species numbers. Species richness is of interest because we may wish to preserve species-rich areas, and also because there may be a link between species richness and community function, as it is generally thought that species-rich communities have characteristics that make them less susceptible to change than species-poor communities Allen (1998).

Plant and animal populations do not operate in isolation. They exist within a community, share the same environments and habitats, and interact with one another in various ways. Within the community, some species may interact more strongly among themselves than with others, utilising habitat or food resources in a similar manner. All communities have certain characteristics that define their biological and physical structure and these characteristics vary in both space and time. Communities are characterised not only by the mix of species (the biological structure), but also by physical features of the biotic and abiotic environment.

Clearly community events result from, and are therefore determined by, biotic processes, such as breeding and competition. However, other aspects of the community are determined by environmental factors such as disturbance

(Connell 1978), temperature (Turner et al. 1987), and exposure (Keddy 1982, 1983), and such factors constrain biotic processes. Studies (Hilborn and Sterns 1982; Fisher and Grimm 1991; Woodward and Hildrew 2002) have led to the conclusion that communities are structured by multiple interactions of organisms with their biotic environment and with abiotic factors as well. Which of these interactions are most important can vary from one type of community to another, and even among different components of the same community (Ricklefs and Schlüter 1993; Begon et al. 1996).

The experimental quantification of community structure, and factors which regulate it, has proved to be very difficult (Fisher 1995; Mduma et al. 1999). It is usually impossible to employ the same method of measurement over the whole community leading to problems of compatibility of measurement within studies

The programme of work presented in this thesis is divided into two distinct parts. The first is largely observational and descriptive and provides information on species composition of the natural plant community within Loch Lomond. This section provides an insight into the level of primary production by aquatic macrophytes in Loch Lomond, describes recent changes in the plant community within the loch as a result of alien introductions, and provides an insight as to the implication of these introductions to the native flora of the loch.

In the second part of the study, further descriptive information is provided on the loch communities, focusing on the benthic macroinvertebrate and fish communities of the loch, in order to identify changes in community structure as a result of biotic and abiotic factors. The implications of community structure on the trophic niche width of a ruffe (*Gymnocephalus cernuus*) population, ubiquitous in Loch Lomond is explored, and a comparison made between traditional

methodology and stable isotope analysis in determining trophic niche width. Finally, the consequences of modified niche width on individual fitness is investigated.

1.1 General Introduction to the Loch Lomond Area

The physical and biological characteristics of Loch Lomond make it the ideal choice of study site for this programme of work. With a surface area of 70.27 km² (Best and Traill 1994), Loch Lomond is Britain's largest area of fresh water. Due to its near north-south orientation, the loch cuts across the main structural trends and geological features of Scotland (Macdonald 1974). The most important of these is the Highland Boundary Fault. This is a geological fault line that transects Scotland from the west to the east coast, marking the transition from the southern edge of the Highlands, where the bedrocks are mainly schist/schistose grits, to the northern edge of the Central Lowlands where the rocks are mainly sandstone (Macdonald 1974). The unique geology of the area means that along its 36.25 km length, the loch is comprised of a chain of largely discrete basins, of increasing width and decreasing depth (Figure 1.1). The northern basin is narrow and steep, reaching a depth of 200m with a breadth of 1.5 km (Tippett 1994). The mid basin, south of Ross Point, opens out to form a wider (3.5 km (Tippett 1994)) and shallower basin with a maximum depth of 66 m (Pomeroy 1994). In the southern basin, the loch is shallow with the depth here rarely exceeding 20m (Pomeroy 1994). It is here that the loch reaches its maximum breadth of 8.8 km and is dotted with islands (Pomeroy 1994).

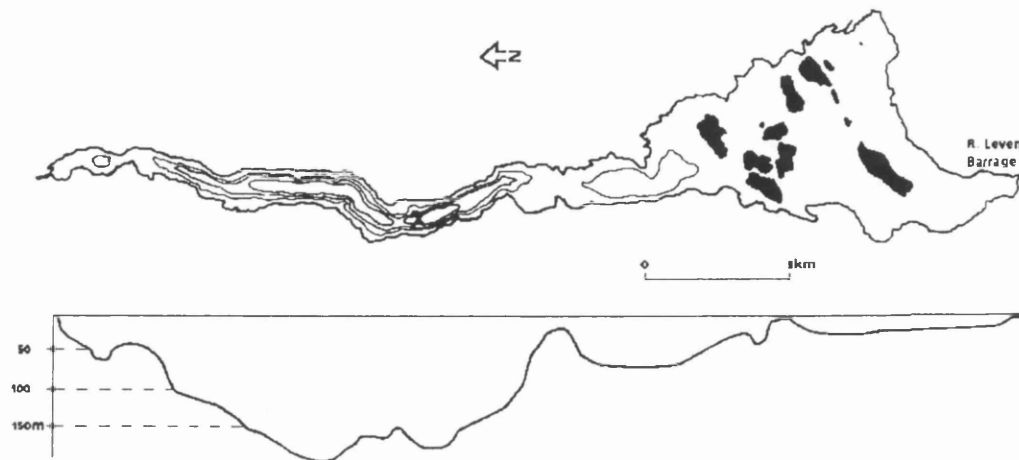


Figure 1.1 Morphometry of Loch Lomond (adapted from Smith et al. 1981) illustrating position of islands (shaded black) and depth contour lines.

The influence of the geology of the catchment area is not restricted to basin topography alone. The southern catchment of Loch Lomond consists of soft sedimentary rocks and fertile soils, which leach soluble minerals into the loch via river inflow and water runoff (Macdonald 1974), while the hard and peat-covered rock of the northern basin contributes very little in the way of minerals to the waters of the loch (Macdonald 1994). Differing land use in these areas adds to the disparity in fertility of the northern and southern basins (Dickinson 1994). The poor soils of the northern catchment are generally used for grazing sheep or forestry purposes, whereas the southern catchment is farmed more intensively, with mineral fertilisers and organic matter applied to enrich the soil in this much more densely populated portion of the catchment (Dickinson 1994).

Being within easy driving distance of the populace of the Central Belt of Scotland, Loch Lomond sees huge numbers of visitors in the summer. The immense popularity of the area results in many forms of stress being put on the loch environment. It is used for recreation, for hydroelectric power and as a potable water supply. In addition, numerous single dwellings, camping and caravan

parks, youth hostels, hotels and small villages discharge effluent into the loch. The continuing pressures placed on Loch Lomond has implications for its fauna and flora and has facilitated the introduction and rapid growth of populations of invasive species of fish (Adams 1994), invertebrate and euhydrophyte (Murphy et al. 1994; Macleod and Murphy 2002). Through this programme of work, the insights gained into the trophic functioning of the loch will enhance our understanding of the manner in which the loch responds to natural and human disturbance. With the designation of Loch Lomond and the surrounding area as Scotland's first National Park from 2002, there is a need for detailed information on the natural resource base of the loch and how it is changing in response to new pressures on the ecosystem. This information is essential if we hope to conserve and enhance the natural resources of the area.

1.2 Food Web Studies

Food webs are typically very complex, and some conceptual simplifications are necessary in order to disentangle them (Vander Zanden et al. 1999). One of these utilised here is the notion that organisms can be grouped together into trophic levels (Schoener 1989; Pimm et al. 1991; Martinez 1994). The assignment of organisms to these trophic levels is determined by the number of energy transfers that they are away from the original source of the energy i.e. photosynthesis (Schoener 1989; Pimm et al. 1991; Martinez 1994). The number of these trophic levels (also known as food chain length) is commonly referred to as the trophic structure of the system.

The trophic level concept has proven useful in studies of trophic cascades (Smith et al. 1981; Power 1990; Mazumder 1994) and continues to provide a framework for models and field studies of food web dynamics, often making these studies

possible by simplifying trophic structure to a manageable form (Vander Zanden et al. 1999).

The trophic structure of a system has implications for both community and ecosystem patterns and processes, such as the regulation of species diversity (Paine 1980) and the biomass of trophic levels (Hairston et al. 1960; Oksanen et al. 1981; Power 1990; Hairston and Hairston 1993; Mazumder 1994; Abend and Smith 1995). The difficulty of determining trophic relationships in natural ecosystems is a major obstacle to our understanding of ecosystem processes, as for example, lakes that are similar in terms of community composition can differ in trophic structure when energy flow is taken in to account (Vander Zanden et al. 1999).

Food chain analysis provides the basis for most studies of food web dynamics, but fails to incorporate the complexity and omnivory that characterises natural ecosystems (Polis and Strong 1996). The approach provides overly simplistic trophic depictions by assuming no omnivory and the existence of discrete trophic levels (Polis 1991; Polis and Winemiller 1996). Another disadvantage is that feeding links are not weighted according to their energetic or functional importance (Vander Zanden and Rasmussen 1996). Presented in this thesis is the theoretical background and empirical evidence to support the theory that stable isotope analysis is a powerful tool to investigate and elucidate trophic structure. The stable isotope approach can provide information on trophic status which is continuous, rather than discrete and also provides an integrated measure of assimilation, making it possible to assess the relative contribution to the food web of resources with distinct isotopic signatures (Lajtha and Michener 1994).

1.3 Stable Isotopes

Atoms consist of a nucleus of protons and neutrons surrounded by a cloud of electrons. An element is defined by the number of protons in the nucleus of the atom. For example, the element carbon has six protons, whereas the element nitrogen has seven. Although the number of protons is fixed for a particular element, the number of neutrons can vary. Carbon can have six, seven or eight neutrons whereas nitrogen can have seven or eight neutrons in the nucleus. The various combinations of protons and neutrons are called isotopes, which are distinguished on the basis of atomic mass (Criss 1999). Atomic mass is the number of protons plus the number of neutrons and therefore the naturally occurring isotopes of carbon are carbon-12 (6 protons + 6 neutrons), carbon-13 (6 protons + 7 neutrons), and carbon-14 (6 protons + 8 neutrons) which are abbreviated as ^{12}C , ^{13}C , and ^{14}C respectively. The naturally occurring isotopes of nitrogen are nitrogen-14 (7 protons + 7 neutrons), and nitrogen-15 (7 protons + 8 Neutrons) and are abbreviated as ^{14}N and ^{15}N respectively. The isotope ^{14}C has an unstable nucleus which is transformed into another configuration without the addition of energy from the outside. ^{14}C undergoes radioactive decay to an isotope of nitrogen (^{14}N). and as a result of this, ^{14}C is called a radiogenic or 'unstable' isotope. In contrast, the stable isotopes of carbon (^{12}C and ^{13}C) and nitrogen (^{14}N and ^{15}N) do not undergo radioactive decay and are called stable isotopes. In the case of both carbon and nitrogen the lighter of the two stable isotopes had a much higher % natural abundance. ^{12}C had a natural abundance of 98.89% while ^{13}C has a natural abundance of 1.11% (Platzner 1987), while ^{14}N has a natural abundance of 99.63% and ^{15}N has a natural abundance of 0.37% (Platzner 1987).

Table 1.1 Nuclear compositions of carbon and nitrogen atoms (adapted from (Holtzclaw et al. 1999))

	Symbol	Atomic number	Number of protons	Number of neutrons	Mass (amu)	% Natural abundance
Carbon	¹² C	6	6	6	12.0000 ^a	98.89
	¹³ C	6	6	7	13.0033	1.11
	¹⁴ C	6	6	8	14.0032	-----
Nitrogen	¹⁴ N	7	7	7	14.0031	99.63
	¹⁵ N	7	7	8	15.0001	0.37

^a mass assigned as exactly 12 by international agreement.

The environmental isotopes such as carbon, nitrogen and hydrogen are the naturally occurring isotopes of elements found in abundance in our environment. These are principal elements of hydrological, geological and biological systems and the stable isotopes of these elements serve as tracers of water, carbon, nutrient and solvent cycling. They are light elements, and as a consequence, the relative mass differences between their isotopes are large (Table 1.1), imparting measurable fractionations during physical chemical and biological processes, resulting in a change in isotopic abundance between chemical species.

Fractionation of isotopes describes a change in the isotopic ratio of a substance. A substance may become partitioned into two or more fractions which have a ratio of 'heavy' to 'light' isotopes, different to that of the initial naturally occurring (geochemical) ratio. Following isotopic analysis, if there is found to be an increase in the heavy isotope when compared to the starting ratio, the sample is considered isotopically enriched through fractionation processes. Conversely, if there is a decrease in the proportion of the heavier isotope, then the sample is considered to be depleted in that isotope due to fractionation processes (Criss 1999).

Two different types of processes – equilibrium and kinetic isotope effects – cause isotopic fractionation. Equilibrium isotopic fractionation occurs among chemical

species linked by equilibria as a result of bond strength differences between the isotopic species (Hayes 1993) while kinetic isotope effects occur because of differences in the rate of transport or rate of reaction of isotopic species (Farquhar et al. 1989; Hayes 1993). Biological processes are generally unidirectional and are kinetic isotope reactions. During these biological processes, organisms preferentially break down and excrete the lighter elemental isotope since the energy required to break these bonds is less. As a result of this, the heavier isotope is retained and incorporated into the tissues and this causes significant and measurable fractions which can be traced through the food web (Rundel et al. 1988; Lajtha and Michener 1994; Abend and Smith 1995; Griffiths 1998).

1.4 Introduction to Stable Isotope Analysis

In general, lighter isotopes tend to form weaker bonds and to react faster than heavier isotopes. As a consequence of these bond-energy and reaction rate differences, the abundance of stable isotopes of an element will vary between chemical species.

Stable environmental isotopes are measured as the ratio of the two most abundant isotopes of a given element. For carbon these are ^{12}C which has an abundance of 98.90% and ^{13}C which has an abundance of 1.10% (Table 1.1). The nitrogen isotopes are ^{14}N , with an abundance of 99.634%, and ^{15}N , with an abundance of 0.366% (Table 1.1). To make measurements of isotopic abundance of a manageable magnitude, because they can be very small, the

isotopic composition of most materials is expressed as the normalised ratio of the sample to a standard, in parts per thousand (per milie, ‰):

$$\delta X = [(R \text{ Sample} / R \text{ Standard}) - 1] * 1000$$

Where R Sample and R Standard are the ratios of heavy to light isotopes for the sample and the standard respectively (Gaston and Suthers 2004). A positive δX value means the sample has more of the heavier isotope than the standard and is referred to as enriched.

The use of natural abundance variations in stable isotopes as tracers relies on the fractionations that occur during chemical, physical and biological processes. Differences in fractionation during these processes lead to distinct “isotopic signatures” for biological materials (Rundel et al. 1988; Ehleringer et al. 1993; Lajtha and Michener 1994; Griffiths 1998). However, there is considerable variation among ecosystems at the base of the food web in the $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{base}}$) and the $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{base}}$) from which organisms draw their nitrogen and carbon (Rounick and Winterbourn 1986; Zohary et al. 1994; Cabana and Rasmussen 1996; Macleod and Barton 1998; Vander Zanden and Rasmussen 1999). Baseline values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ show spatial and temporal variation both within a lake (France 1995; Vander Zanden and Rasmussen 1999) and among lakes (Cabana and Rasmussen 1996; del Giorgio and France 1996) and as a result the stable isotope signature of an organism alone provides little information about its absolute trophic position or source of carbon since without a suitable estimate of $\delta^{13}\text{C}_{\text{base}}$ and $\delta^{15}\text{N}_{\text{base}}$ there is no way to determine if variation in the isotopic signature of an organism reflects changes in food web structure and carbon flow or if it is simply a result of differences in baseline isotopic signature. Therefore,

when conducting a study such as this, it is essential that baseline isotopic signatures are taken into account.

1.4.1 Nitrogen Isotopes

The abundance of ^{15}N in the tissues of a consumer is typically enriched over the level found in their prey due to the preferential excretion of the lighter isotope during metabolic processes (Macko et al. 1982; Minagawa and Wada 1984; Peterson 1999). This means that there is a detectable isotopic enrichment in the ^{15}N of animal tissues relative to their food source and this can be used to indicate trophic position (Doucett et al. 1996). The $\delta^{15}\text{N}$ of a consumer is typically enriched by 3.4‰ relative to its diet (DeNiro and Epstein 1981; Minagawa and Wada 1984; Peterson and Fry 1987; Post 2002). In order to determine trophic position accurately using stable isotopes, it is important to interpret the $\delta^{15}\text{N}$ of a consumer relative to an appropriate baseline as this provides a continuous energy flow-based measure of the mean number of transfers between the producer organisms and the species under investigation (Vander Zanden et al. 1999). During the course of the study, $\delta^{15}\text{N}$ values were determined as a relative measure of niche width within a single community and this measure was compared between communities.

The nitrogen isotopic content of an organism does not reveal which prey species are consumed by it but has the advantage of taking into account not just ingested food, but food that is actually assimilated (Peterson and Fry 1987; Kling et al. 1992; Cabana and Rasmussen 1996). Bearing in mind that species composition and gut content analysis provide only indirect information on trophic interactions,

which may be misleading if assimilation is not considered (Grey et al. 2002), it is understandable that stable isotopes are increasingly used in food web studies (Peterson and Fry 1987). Stable isotope analysis has proved to be particularly useful when combined with conventional dietary analysis (Hobson and Welch 1995; Vander Zanden et al. 1997; Beaudoin et al. 2001; Grey 2001) and this concept was used to elucidate the food web structure in Loch Lomond.

1.4.2 Carbon Isotopes

Carbon atoms occur in three different masses, or isotopes (see Table 1.1). Unlike high temperature processes in deep earth, low temperature biological processes such as photosynthesis are sensitive to these differences in mass and actively filter different carbon isotopes (Park and Epstein 1960; Keeley and Sandquist 1992). Thus the ratios of carbon isotopes in organic materials, plants, animals and shells, vary and are also measurably different from those in the carbon dioxide of the atmosphere and the oceans (Keeley and Sandquist 1992).

The ratio of carbon isotopes ($\delta^{13}\text{C}$) within tissues changes little as trophic level increases and carbon moves through the food web (Rounick and Winterbourn 1986; Peterson and Fry 1987). As a result of this, ^{13}C is a useful indicator of sources of production since differential fractionation of stable isotopes of carbon in aquatic and terrestrial plants can cause primary producers to have distinct carbon isotope signatures (Griffith 1992; Keeley and Sandquist 1992). The differences in diffusional resistance between terrestrial and aquatic systems (with resistance being orders of magnitude greater in the aquatic environment) means that in aquatic systems there is reduced mixing of the carbon pool in the

boundary layer with that of the bulk solution and as a result aquatic plants are forced to draw from a finite carbon pool, resulting in a reduction of discrimination against the heavier isotope. Terrestrial C_3 plants have a $\delta^{13}C$ between -20 and -25‰ whereas aquatic C_3 plants are markedly less negative. Because $\delta^{13}C$ values are conserved up the food chain but vary at the base of the food chain, the $\delta^{13}C$ of aquatic consumers can provide information about the sources of energy exploited by higher consumers (Rounick and Winterbourn 1986; Peterson and Fry 1987).

The $\delta^{13}C$ content of components of fresh waters can vary widely depending on the source of dissolved carbon in the water, with a value of $+1\text{‰}$ where the carbon source is present in the form of HCO_3^- derived from limestone to approximately -7‰ for CO_2 dissolved in air-equilibrated water (Keeley and Sandquist 1992). Where source carbon is derived autochthonously, through respiration of aquatic flora or fauna, or allochthonously through decomposition of litter input to the system, $\delta^{13}C$ values can be as low as -30‰ (Keeley and Sandquist 1992; Ehleringer et al. 1993; Gannes et al. 1998).

These concepts were used to investigate the functioning, regulation and status of the aquatic food web in Loch Lomond.

1.5 Project Aims

The main aims of the project were to investigate the mechanisms of energy transfer through trophic cascades in lakes, in order to determine the role of community structure in modulating trophic niche position and width for an individual species, and to determine the consequences for individual organisms of modified trophic niche. Specifically, traditional dietary analysis was used to

describe and quantify the degree of interaction in contrasting food webs from six sampling sites in Loch Lomond. The mean value of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between species pairs was used as an index of the potential for competition. These indices were calculated for a ubiquitous species (ruffe) in six discrete communities within Loch Lomond, in order to test hypotheses on the effect of food chain complexity on trophic position and niche breadth. The consequences of changes in trophic position and niche breadth on the fitness of individual organisms were examined by determining fat deposition rate and growth of organisms with differing trophic status.

In chapter 2, the role of the macrophyte community as a primary producer in Loch Lomond is examined, and the historical changes which have taken place within the macrophyte community of the loch are described.

In chapter 3, the degree of variation in the community structure is examined at six sampling sites in Loch Lomond. It is postulated that the observed variability in community structure may be driving variation in trophic niche width in ruffe.

In chapter 4, an argument is put forward for the use of the standard deviation of nitrogen stable isotope signatures as a measure of trophic niche width. A test of this hypothesis is made using gut content analysis.

In chapter 5, the physical and biotic drivers of variation in niche width are examined for ruffe at 6 sites in Loch Lomond. The consequences of variations in trophic niche width for individuals within a population are explored.

Chapter 2. Temporal and Spatial Variation in Submerged Macrophyte Communities of Loch Lomond, Scotland

Submerged macrophyte biomass values were assessed monthly from May to October 2001. Samples were taken from 12 sites, 4 from within each of the three basins of Loch Lomond to meet the following aims.

- The data were used to identify macrophyte communities present, dominant species, and seasonal variation within and between basins.
- A comparison was made with data from a similar survey carried out in 1990 and this information was used to assess longer-term changes in the macrophyte communities of Loch Lomond.
- The invasion of non-native species and changing nutrient levels were identified as threats to the macrophyte communities of Loch Lomond.

This chapter has been published as a paper: Macleod, H & Murphy, K.J. (2002) Temporal and distributional variation in submerged macrophyte communities of Loch Lomond, Scotland. Proc 11th EWRS International Symposium on Aquatic Weeds, Moliets, France, 39-42 (Appendix 1).

2.1 Introduction

Loch Lomond supports a wide range of freshwater plant communities located along a unique gradient of environmental conditions (Murphy et al. 1994). Measuring 70 km², the loch is Britain's largest area of fresh water (Best and Traill 1994; Pomeroy 1994) and the catchment area includes some of the most attractive and easily accessible scenery in Western Europe (Dickinson 1994). The loch is located near Glasgow, the largest centre of population in Scotland, and more than half the population of Scotland live within one hour's journey of the shores (Tippett 1994). As a result of this, Loch Lomond experiences heavy recreational use (Adams 1993), is used as a hydroelectric power source and also as a potable water supply (Dickinson 1994). On the 19th of July 2002, the Loch Lomond and Trossachs National Park, the first of its kind in Scotland, became fully operational. Loch Lomond is the centrepiece of the National Park, and the National Park Authority is required to prepare a National Park Plan, which will set out how the authority will manage the National Park to achieve its aims in the coming years. As a direct result of this designation, new legal restrictions are likely on planning, water quality protection and recreational use in Loch Lomond. In this context there is a need for specific information on the natural resource base of the loch, and how it is changing in response to new pressures on the ecosystem.

Macrophytes are an important component of the aquatic ecosystem. Their physical structure plays a large role in determining fish, zooplankton, and benthic habitats in the littoral zone of lakes, influencing food web structure (Cyr and Downing 1988; Beklioglu and Moss 1996; Diehl and Kornijów 1998; Jeppesen et al. 1998). Broad changes in the abundance of individual species and community compositions provide valuable information on how and why an ecosystem might

be changing (Palmer 2001). Macrophytes are also becoming increasingly valued as a means of indirectly monitoring water quality, as for instance, eutrophication can produce a progressive change in species composition and a loss of species diversity (Rørslett et al. 1986; Blindlow 1992).

The data gathered here were used to identify macrophyte communities present, to explore differences in macrophyte production between basins and to examine the possible consequences of eutrophication (Best and Traill 1994) on the submerged macrophyte communities. In order to assess long-term trends of change within the loch system, a comparison was made with a previous survey of Loch Lomond carried out in 1990 (Murphy et al. 1994).

2.2 Methods

Monthly surveys of submerged macrophyte biomass were carried out in the three basins (South, Mid and North) of Loch Lomond during May to October 2001 (see table 2.1 for survey dates). Four sites with varying intensities of wind exposure were selected from each basin (Figure 2.1). Spence (1964) calculated that up to 20% of the total surface area of Loch Lomond was suitable for plant growth. This corresponds approximately with the 10m-depth contour of the loch (Figure 2.3). Of this area, he estimated that only 1% is actually colonised by plants, probably due the high levels of unsuitable substrates such as rippled sand found within the loch (Spence 1964). With this in mind, sites were chosen to ensure that sampling was carried out at sites where conditions were suitable for macrophyte growth.

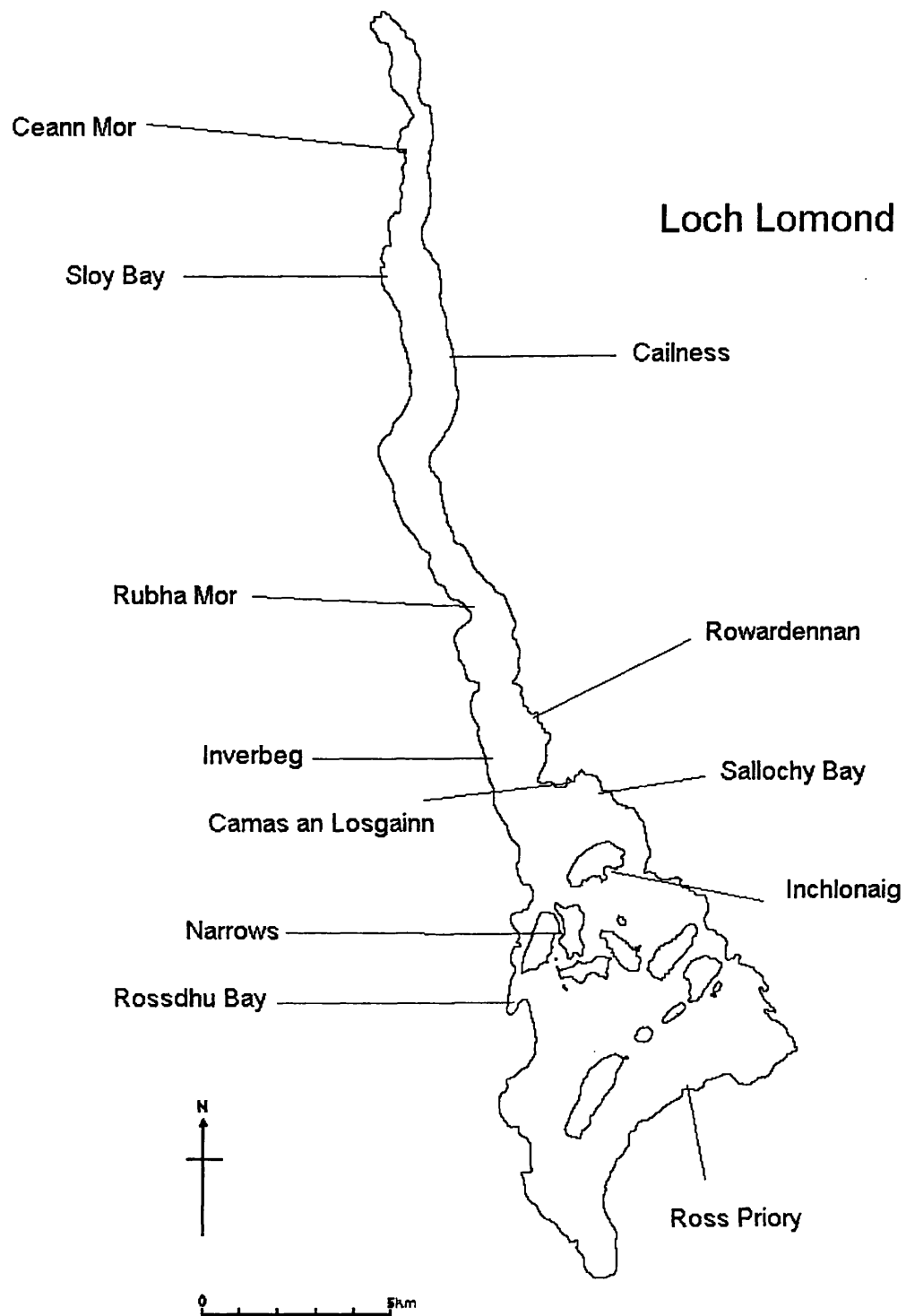


Figure 2.1 Loch Lomond map illustrating 2001 sampling sites.

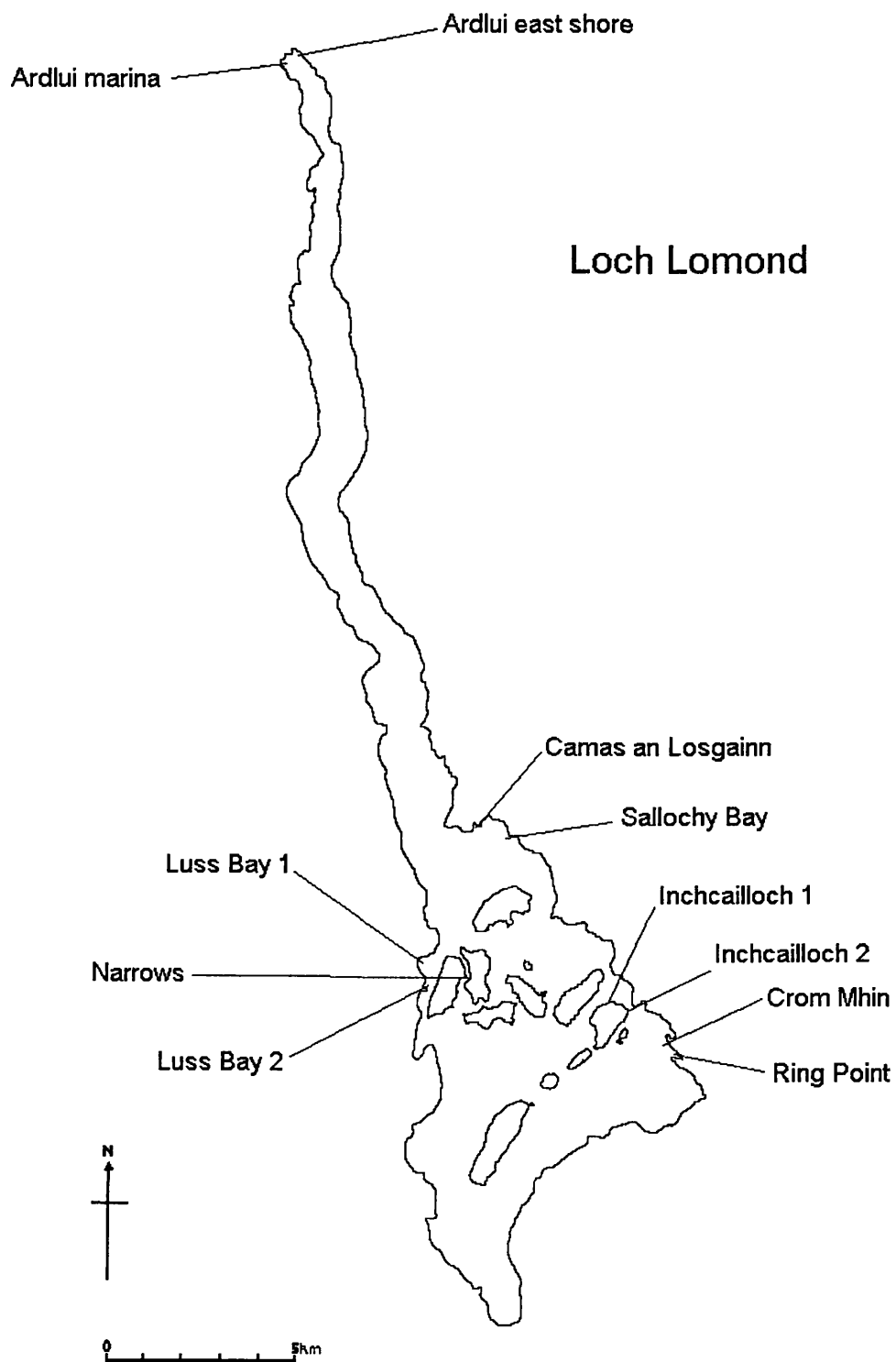


Figure 2.2 Map of Loch Lomond illustrating 1990 sampling sites.

From each of the sampling sites, three Ekman grab samples (area 0.155m x 0.155m) were taken. All plant material within the sample was collected, washed, identified to species, and dried at 60°C prior to weighing.

Table 2.1 Details of macrophyte sampling dates, number of sites sampled and the number of samples taken.

Date of Survey	Number of sites sampled	Total number of samples
24 May 2001	12	36
21 June 2001	12	36
20 July 2001	12	36
10 August 2001	12	36
30 August 2001	12	36
15 October 2001	12	36

Data collected in 2001 were compared to that collected in 1990 by Murphy et al (1994). Figure 2.2 illustrates 1990 sampling sites. TWINSpan (Hill 1979) analysis was used in the classification of this data into groups. TWINSpan provides a hierarchical divisive classification of the data matrix (Gauch 1982), classifying both samples and species and constructing an ordered two-way table which expresses succinctly the relationships of samples and species within the data set. It also identifies 'indicator species' which separate the sample groupings at each level of division. This allows the classification of species onto groups and this was carried out on combined 1990 and 2001 data to identify trends of change in the macrophyte communities of Loch Lomond over the 11 year period between these two surveys.

2.3 Results

During the course of the 2001 study, twelve species of aquatic macrophyte were identified in Loch Lomond (Table 2.2). Of these twelve species, ten were native and two were invasive species. Both of the invasive species recorded were of the genus *Elodea*, *Elodea nuttallii* and *Elodea canadensis*, and contributed a total biomass of 3.19g/m² dry weight, which accounts for 5.76% of the total biomass recorded. *Littorella uniflora*, which is native to Loch Lomond, was the most common species recorded and contributed over 40% (22.48g/m²) of the total macrophyte biomass in Loch Lomond (Table 2.2).

Table 2.2 Macrophyte species, status and average biomass (g dry weight/m²) recorded for Loch Lomond over the period May to October 2001.

Species	Status	Average biomass (g/m ² dry weight)	% Biomass
<i>Littorella uniflora</i>	Native	22.4796	40.6338
<i>Isoetes lacustris</i>	Native	15.0531	27.2097
<i>Myriophyllum alterniflorum</i>	Native	9.2741	16.7637
<i>Elodea nuttallii</i>	Invasive	3.0174	5.4542
<i>Nitella flexilis</i>	Native	2.0858	3.7702
<i>Lobelia dortmanna</i>	Native	1.5132	2.7353
<i>Juncus bulbosus</i>	Native	1.4023	2.5348
<i>Potamogeton perfoliatus</i>	Native	0.2359	0.4264
<i>Elodea canadensis</i>	Invasive	0.1682	0.3041
<i>Callitriche hamulata</i>	Native	0.0879	0.1590
<i>Fontinalis antipyretica</i>	Native	0.0045	0.0081
<i>Potamogeton friesii</i>	Native	0.0005	0.0009

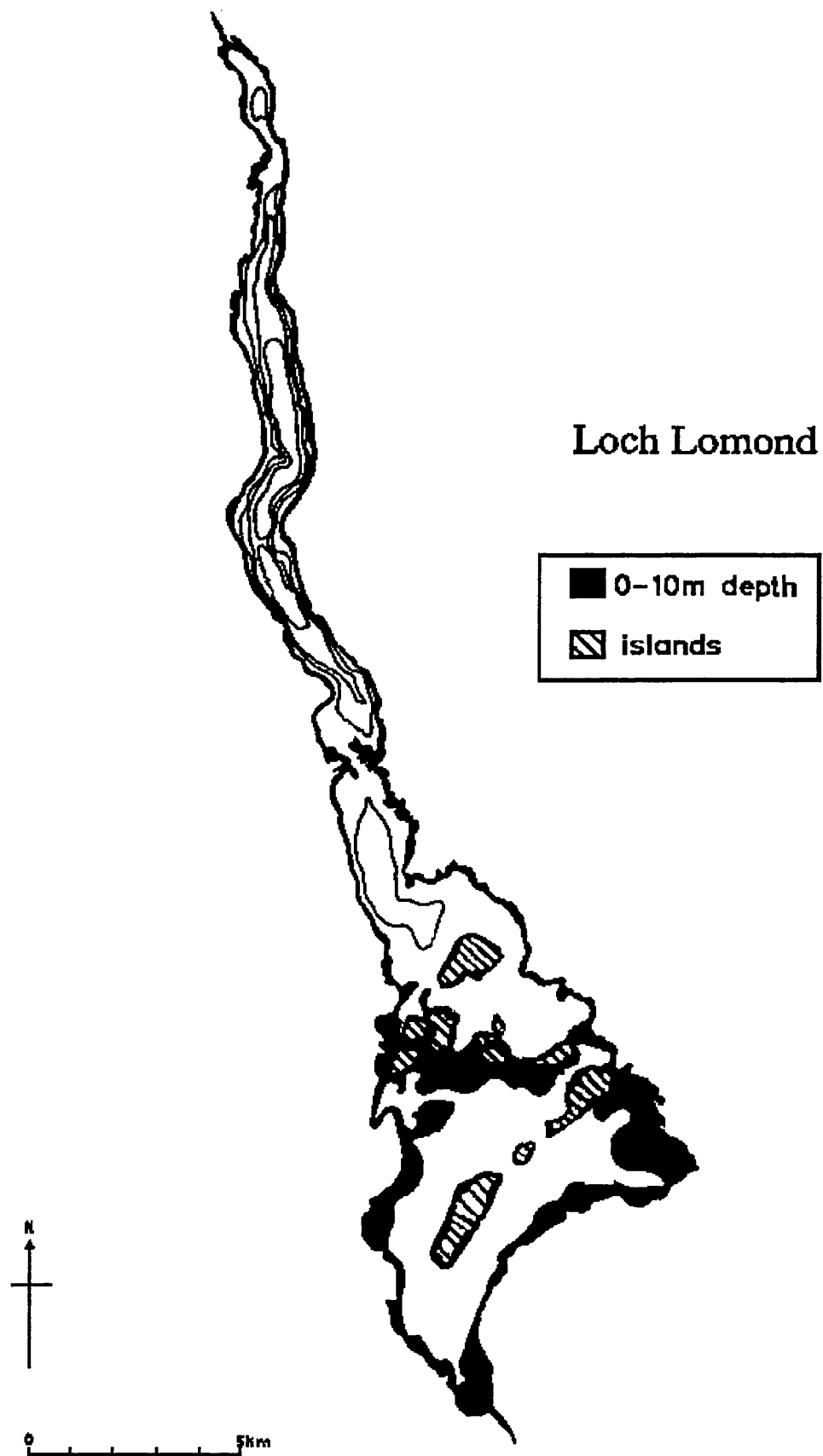


Figure 2.3 Distribution of the approximate euphotic zone: 0 - 10m in Loch Lomond (adapted from Murphy *et. al.* 1994).

There were differences in the values of macrophyte biomass recorded within each basin of Loch Lomond (Figure 2.4). The mid basin of the loch had the least macrophyte growth and this was true for each of the species identified within the mid basin. Levels of macrophyte growth were similar within the north and south basins of Loch Lomond, however there are many more sites which are suitable for macrophyte growth within the south basin than in the north basin of the loch (Figure 2.3) and as such overall basin macrophyte growth can be considered to be at its highest within the south basin of Loch Lomond.

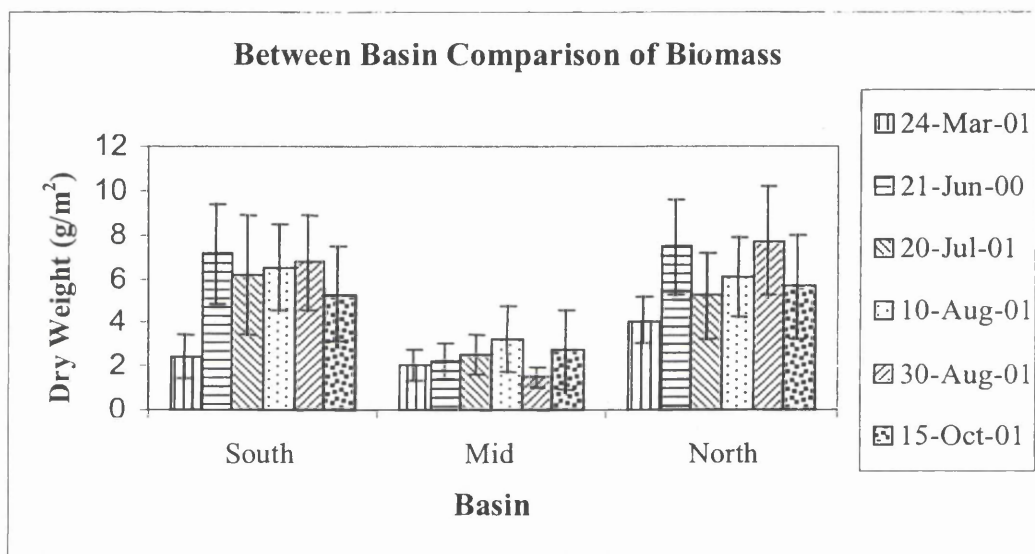


Figure 2.4 Between basin comparisons of average monthly macrophyte biomass (g dry weight per m² \pm standard error)

Comparison of 1990 and 2001 data using TWINSpan analysis identified three main community types, labelled A, B and C in Table 2.3. Group A comprises the two sites with the highest number of species recorded, both of which are 1990 sites. This group contains 20 of the 25 species recorded over the course of the 1990 and 2001 studies. The indicator species for group A are *Utricularia* spp. Group B is made up entirely of 2001 sampling sites and comprises 9 species. This group has two indicator species, *Elodea nuttallii* and *Lobelia dortmanna*. The

remaining sites which, contain 12 species, make up group C, with *Elodea canadensis* and *Potamogeton perfoliatus* as indicators.

Table 2.3. Table of sites x species produced by TWINSpan for the combined 1990 and 2001 Loch Lomond vegetation data sets. Groups A-C are shown with indicator species underlined.

Groups:	B	C	A
Sites:	11	1222 112111	11
	0217839	52690134562178	34
<i>Elodea canadensis</i>	-1-----	- <u>111111</u> - <u>111</u> ---	11
<i>Persicaria amphibian</i>	-----	-----1-----	--
<i>Potamogeton friesii</i>	-----	1-----	--
<i>Potamogeton perfoliatus</i>	1-----	<u>1-11</u> --- <u>1-1</u> -- <u>1</u>	--
<i>Ranunculus peltatus</i>	-----	-----1-----	--
<i>Isoetes lacustris</i>	1111111	1111-111111---	11
<i>Littorella uniflora</i>	1111111	111111111111-11	11
<i>Myriophyllum alterniflorum</i>	11-1111	111111111111--	11
<i>Nitella flexilis</i>	-1--111	-----11111111	1-
<i>Elodea nuttallii</i>	<u>111</u> --11	111-----	--
<i>Lobelia dortmanna</i>	<u>11111</u> --	-----	11
<i>Fontinalis antipyretica</i>	-----	-----1-----	-1
<i>Apium inundatum</i>	-----	-----	1-
<i>Carex</i> sp.	-----	-----	-1
<i>Hydrocotyle vulgaris</i>	-----	-----	-1
<i>Juncus acutiflorus</i>	-----	-----	-1
<i>Sparganium angustifolium</i>	-----	-----	-1
<i>Eurynchium praelongum</i>	-----	-----	-1
<i>Sphagnum cuspidatum</i>	-----	-----	-1
<i>Sphagnum subsecundum</i>	-----	-----	-1
<i>Sphagnum</i> sp.	-----	-----	-1
<i>Utricularia</i> sp.	-----	-----	<u>11</u>
Leafy liverwort	-----	-----	-1
<i>Juncus bulbosus</i>	-----	-----1--	11
<i>Callitriche hamulata</i>	11-----	-----	11

Note: [-] = absent from site; [1] = present in the site; numbers in **bold** represent 2001 sampling sites (site codes: read downwards). Sample codes: South basin 1990:15 16 17 18 19 20 & 21; 2001:1 2 3 & 4; Mid basin 1990:22 & 23; 2001:5 6 7 & 8; North basin 1990:13 & 14; 2001:9 10 11 & 12.

2.4 Discussion

Loch Lomond supports a wide range of freshwater plant communities located along gradients of conditions from oligotrophic to eutrophic (Murphy et al. 1994). Eutrophication (Best and Traill 1994) is an increasing threat to these aquatic macrophytes and may facilitate the growth and spread of invading nuisance species adapted to richer nutrient conditions such as *Elodea* spp. which may out compete and exclude native submerged species adapted for growth in an oligotrophic-mesotrophic waterbody. During their study of the macrophyte community of Loch Lomond, Murphy et al. (1994) reported that within the south basin of Loch Lomond during 1992-1993, *Littorella uniflora* and other isoetid plants were covered by dense growths of epiphytic algae. However, Marrs (1994), who carried out a survey of the loch in 1991, found little evidence of epiphytic algal growth on *Littorella* from the same area. This requires further investigation as increases in epiphytic algae are a well-known warning sign of eutrophication (Phillips et al. 1978). Further evidence of the growing threat of eutrophication in the southern basin of Loch Lomond is contained within a report published by the Scottish Environment Protection Agency on Phosphorus Control in Loch Lomond (SEPA 2000). The report states that phosphorus levels in the southern basin of the loch have increased from 8.8 $\mu\text{g/l}$ in 1995 to 11.6 $\mu\text{g/l}$ in 2000. Predictive models have suggested that phosphorus concentrations have risen over the last 60-140 years (SEPA 2000a), and further evidence of these increasing phosphorus levels comes from palaeolimnological studies on diatom remains in sediment cores and from phytoplankton monitoring (SEPA 2000b).

The results demonstrate that, as in 1990 (Murphy et al. 1994), *Littorella uniflora* remains the dominant species in Loch Lomond, followed by *Isoetes lacustris* and *Myriophyllum alterniflorum* (Table 2.2). During their 1990 survey, Murphy et al.

(1994) recorded the spread of *Elodea canadensis*, thought to have invaded Loch Lomond between the time of Idle's survey in 1967 (summarised by Bailey-Watts and Duncan 1981) and a Glasgow University field course in 1988, when it was one of the common species recorded. Since 1990, a second invasive species, *Elodea nuttallii*, has colonised the loch (Macleod and Murphy 2002). *Elodea nuttallii* is now present throughout the length of Loch Lomond and in the relatively short space of time since its appearance (some time between 1990 and 2001) has spread rapidly and is now the fourth most common macrophyte species found in the loch (Macleod and Murphy 2002). Comparison of 1990 and 2001 data indicate that *E. nuttallii* and *E. canadensis* serve as indicator species for different community types. This is significant since it was initially thought that *E. nuttallii* would outcompete and replace *E. canadensis* throughout Loch Lomond and as yet this has not taken place.

Spence (1964) calculated that up to 20% of the total surface area of Loch Lomond was suitable for plant growth. This corresponds approximately with the 10m-depth contour of the loch (Figure 2.3). Of this area, he estimated that only 1% is actually colonised by plants, probably due the high levels of unsuitable substrates such as rippled sand found within the loch (Spence 1964). The majority of areas suitable for plant growth lie within the South basin. Data collected in 2001 (Figure 2.4) suggest that biomass values are lower in the mid basin of Loch Lomond, possibly due to higher shoreline exposure in this area. The South and North basin sampling sites were similar in levels of macrophyte production although areas suitable for macrophyte colonisation were greatly lower in the North basin compared to the South basin (Figure 2.3).

The introduction of non-native aquatic macrophyte species, coupled with changing nutrient levels, pose a very real threat to the euhydrophyte communities

of Loch Lomond. Increasing nutrient levels have the potential to facilitate the growth and spread of invading species adapted to richer nutrient conditions. These may out compete and exclude native submerged species such as *Littorella* and *Isoetes lacustris* which are adapted for growth in oligotrophic-mesotrophic conditions (Murphy et al. 1994). Significant changes have already taken place and although it is almost impossible to prevent the introduction of non-native species to the loch, the question of eutrophication is one which must be addressed.

Chapter 3. Variation in Littoral Community

Structure Between Sites In Loch Lomond

In this chapter the degree to which the invertebrate and fish community structure varies at sites across Loch Lomond was explored to determine whether:

- The loch shows habitat heterogeneity at small spatial scales.
- Diversity was manifest as variability in the invertebrate and fish community structure within the littoral zone.
- For a single fish species (the ruffe - *Gymnocephalus cernuus* L.) there was between-site variability in a number of aspects of its ecology, at relatively small spatial scales (e.g. diet, niche width).

It is suggested that variability in community structure between littoral sites in Loch Lomond may be driving variation in trophic niche width in ruffe. This is explored further in chapters 4 and 5.

3.1 Introduction

The general approach adopted in this study is designed to look for community effects on the feeding web structure, and specifically on the trophic responses in a single target species, the ruffe *Gymnocephalus cernuus*. By comparing animal communities at a number of sites within a single, highly heterogeneous lake, it may be possible to tease apart the effect of variations in community structure and the trophic response of ruffe to this variation. However, in temperate climates such as Loch Lomond, seasonality plays a large role in determining community

structure (Keast, 1979). Within this study, sampling took place in summer and winter to establish any seasonal differences in community structure which may be evident within sampling sites.

Standing waters can be divided into horizontal zones based on factors such as photosynthetic activity, fluctuating water levels and the action of wind generated waves. The littoral zone, or shallow water zone, is the area in which light is able to penetrate to the bottom. The littoral zone of lakes are extremely diverse even within one lake (Maitland 1981) and it is within this zone that the greatest number of habitats are found. Littoral substrates range from bare rock to boulders, stones, gravels, sands, fine clays or organic muds. The effect of water movement in the littoral zone is essential in determining which type of substrate is present and which organisms are capable of growth in such an area.

Within an organism, different tissues will have different turnover times and in the case of stable isotope analysis, will integrate information on dietary preferences over different temporal scales (Hobson and Clark 1992; O'Reilly and Hecky 2002), producing an average ratio related to tissue turnover rate and the life of the organism. Liver has a fast turnover time of only days (Hesslein et al. 1993) and in fish, provides the best isotopic indicator of recent diet. Turnover time for white muscle tissue is on an intermediate time scale (Hesslein *et al.*, 1993; (Tieszen et al. 1983) while bone tissue will integrate dietary information over the longest time scale (Schoninger and DeNiro 1984; Sholto-Douglas et al. 1991) making it possible to track dietary preferences of each individual over time.

A prerequisite for this type of study is that local study areas are significantly different in a range of characteristics that may influence the trophic position of the

focal species. Thus the basic general hypothesis to be tested here was that there was significant variation in a number of specific community structure variables.

3.2 Choice of Study Sites

Based upon physical properties, six sampling sites were selected from Loch Lomond in order to encompass the wide range of littoral habitats available within the loch. The properties of the sites are summarised in Table 3.1 and the location of sampling sites are illustrated in Figure 3.1.

In the lacustrine environment, wind is the most important forcing factor for wave activity (Keddy 1982). An index of exposure was calculated for each study site based on fetch and direction/velocity of wind using the formula previously successfully used in lake vegetation studies by Keddy (1982), Weisner (1987) and Murphy et al (1994):

$$E_E = \sum^4 [exceedance_{90} \times fetch_{90}]$$

Where:

- E_E is the exceedance exposure index,
- $Exceedance_{90}$ is the % of time during which winds of greater than 4.0ms^{-1} velocity blew onto the shoreline at the sampling point per 90° quadrant of the compass rose. Modelled data for Loch Lomond was used here.

- fetch_{90} is the mean fetch (in km) per quadrant, visible from the shoreline sampling point.

Exceedance values were calculated for each site using synthetic wind data generated by models developed for Loch Lomond (EU EUROLAKES project – www.eurolakes.com). This permitted much more accurate calculation of exposure values than had been possible previously using meteorological wind data from fixed points. This is because these fixed points are usually relatively distant from actual biological sampling points (in the case of Loch Lomond the closest available fixed point wind data was that recorded at Glasgow airport) and takes no account of local variation in wind due to factors such as small-scale topography. Average fetch per quadrant was calculated as the mean distance to the opposite shore, or visible land, for quadrants containing open water. At least four fetch values were measured per quadrant, but where the shoreline was complex, or islands were visible from the sampling point, up to four more measurements were made depending on the complexity of the shoreline. All fetch distances were measured in mm on a 1:25,000 map and converted into km.

With an exceedance value of 111.5, Ross Priory in the south basin was the most exposed of all the sampling sites. This site also had the highest recorded pH values. The sampling site at Inchlonaig, located in a small bay on the south shore of the island, was the most sheltered of the sampling sites and had an exceedance value of only 20. Inchlonaig had the highest phosphate and Biological Oxygen Demand (BOD) levels (Table 3.1). A general trend of increasing pH, BOD and phosphate levels was observed with the transition from the north to the south basin of Loch Lomond (Table 3.1).

Table 3.1. Physical characteristics of sampling sites. Sites are ordered north to south. O₂ saturation, BOD, ammonia and phosphate (measured as total phosphate) data were bimonthly mean values provided by the Scottish Environment Protection Agency for the period 1995-2002.

	Inverhoulin	Rubha Mor	Ross Point	Sallochy Bay	Inchlonaig	Ross Priory
NGR	NN 3330 0610	NN 3471 0006	NS 3677 9545	NS 3892 9491	NS 3835 9322	NS 4124 8772
Exceedance exposure index (EE)	58.98	51.2	46	86.4	20	111.5
Distance from south shore (km)	18.38	12.34	7.73	7.19	5.50	0
Slope (horizontal / vertical)	1 in 5	1 in 7	1 in 14	1 in 8	1 in 11	1 in 63
Shore type	Rock shore with hard protection	Rock shore with hard protection	Sandy exposed site	Vegetated beach	Sheltered SE facing rock shore dominated by silt	Protected shore with fine gravel / silt
pH	6.7	6.7	6.8	7.0	7.2	7.3
O₂ % saturation	102.8	101.8	105.2	103.3	83.6	99.8
BOD (mg/L)	0.78	0.72	0.83	0.89	1.8	1.5
Ammonia (mg/L)	0.032	0.020	0.020	0.020	0.027	0.020
Phosphate (mg/L)	0.0039	0.0025	0.0026	0.0040	0.010	0.0083

3.3 Methods

3.3.1 *Field sampling methods*

Fish collection

Fish were collected from each of 6 littoral study sites around Loch Lomond (Figure 3.1). Samples were collected seasonally, in winter (March and April 2002) and in summer (August and September 2002). Table 3.2 lists fish sampling dates. In all cases, nets were set on the bottom in the late afternoon, between 4-6 pm, and retrieved the following morning between 9 and 11 am. Initially four gill nets were set at each site (two nets of 0.8m x 21m, with a mesh size of 21mm; one net of 0.8m x 20m, with a mesh size of 21mm; and one net of 1.5m x 25m, with a mesh size of 23mm). In the event that the number of fish caught was below that required for the study (a minimum of 10 ruffe), more gill nets were reset at the same site the following day. If after a number of attempts, the number of fish caught in the nets remained below ten, fyke nets were also set. These were left *in situ* for a period of up to 7 days. At one site, Ross Priory, supplementary fish were also obtained from the water pumping station on the south shore of Loch Lomond (Figure 3.1). Fish trapped on band screens of 8mm mesh size were removed daily and frozen. The fish caught on the screens at the pumping station and by fyke net do not accurately reflect their relative abundance within the community (Adams and Maitland 1998) and were not used to establish community structure, but provided supplementary biomass of fish where required.

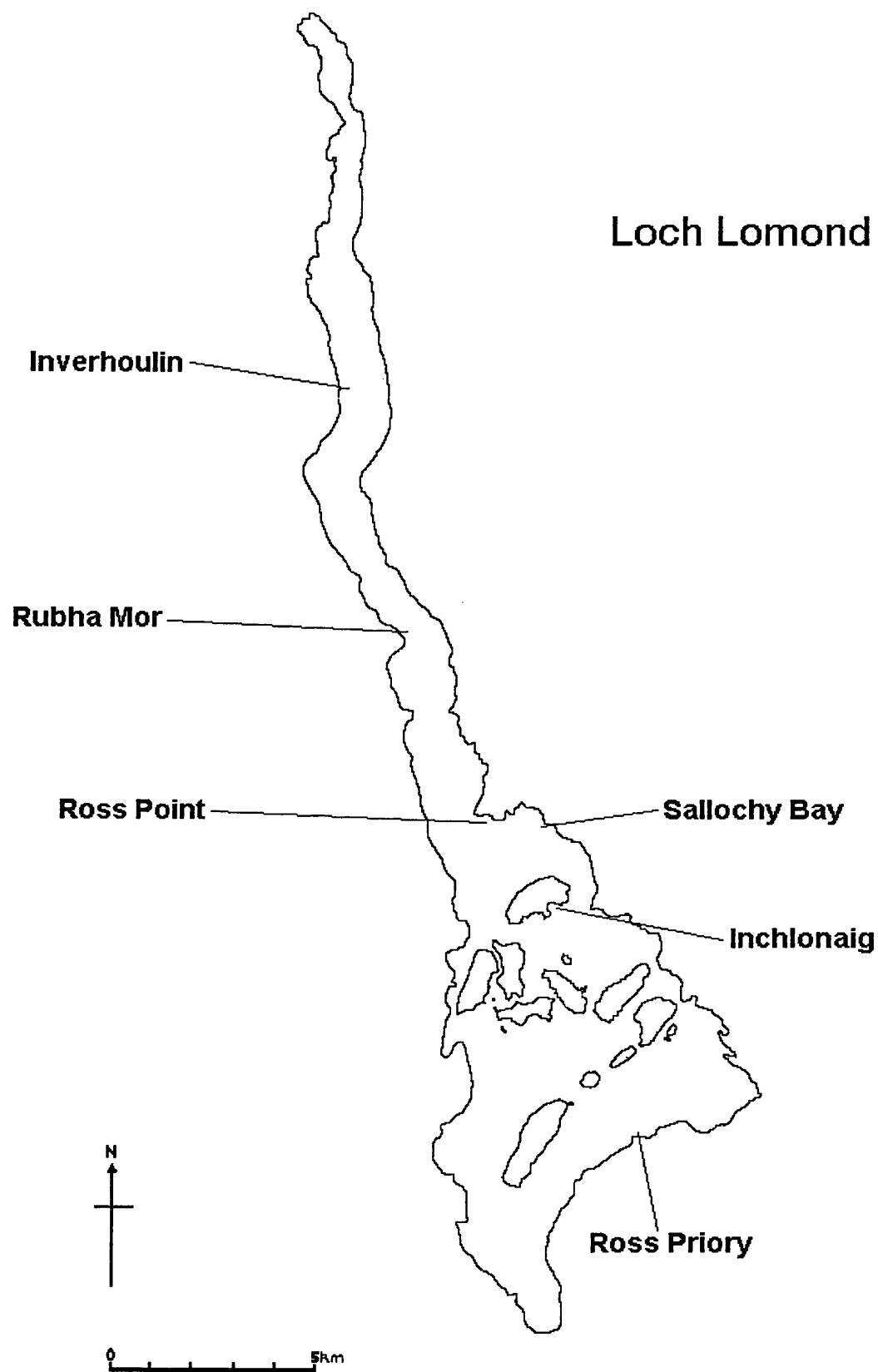


Figure 3.1 Map of Loch Lomond illustrating the location of sampling points.

Invertebrate collection

Benthic invertebrate samples were collected from the littoral zone of 6 sites within Loch Lomond. Invertebrates were collected during the same sampling periods and at the same sites as fish. There are various recognised methods for the sampling of invertebrates such as kick and grab sampling. These methods vary in their effectiveness, depending on the physical conditions in the area to be sampled (Mackey 1972; Pearson et al. 1973). In this study, samples were taken

Table 3.2 Fish sampling dates. * indicates dates where fyke nets were in place, gill nets were used on all other sampling dates.

	March	April	August	September
Inverhoulin	18-19 19-20		14-15 15-16	
Rubha Mor	18-19 19-20 28-29	4-9* 9-17* 17-25*		10-11 11-12 28-29
Ross Point	12-13			11-12 12-13
Salloch Bay	29-30			17-18
Inchlonaig	20-21 21-22	1-6 4-9 9-17*		13-14
Ross Priory	20-21 21-22	3-4 6-7		14-15 16-17

using a modified airlift sampler developed as part of the study. Airlift samplers have an advantage over other sampling devices in that they can operate over a wider range of substrate types compared to alternative methods (Mackey 1972; Pearson et al. 1973). This allowed for the same sampling system to be used over

the wide range of substrate types encountered within sampling sites in Loch Lomond.

The airlift sampler consisted of a 44.5cm length of curved PVCu pipe with a diameter of 15.5cm (Figure 3.2). The weights attached to the sampler ensure that the base of the sampler lies flat on the substrate giving an enclosed sampling area of 189 cm² and allowing quantitative samples to be obtained from a very specific area. Compressed air from an air cylinder was directed down into the substrate by two copper pipes. This blast of air disturbed the sediment, which

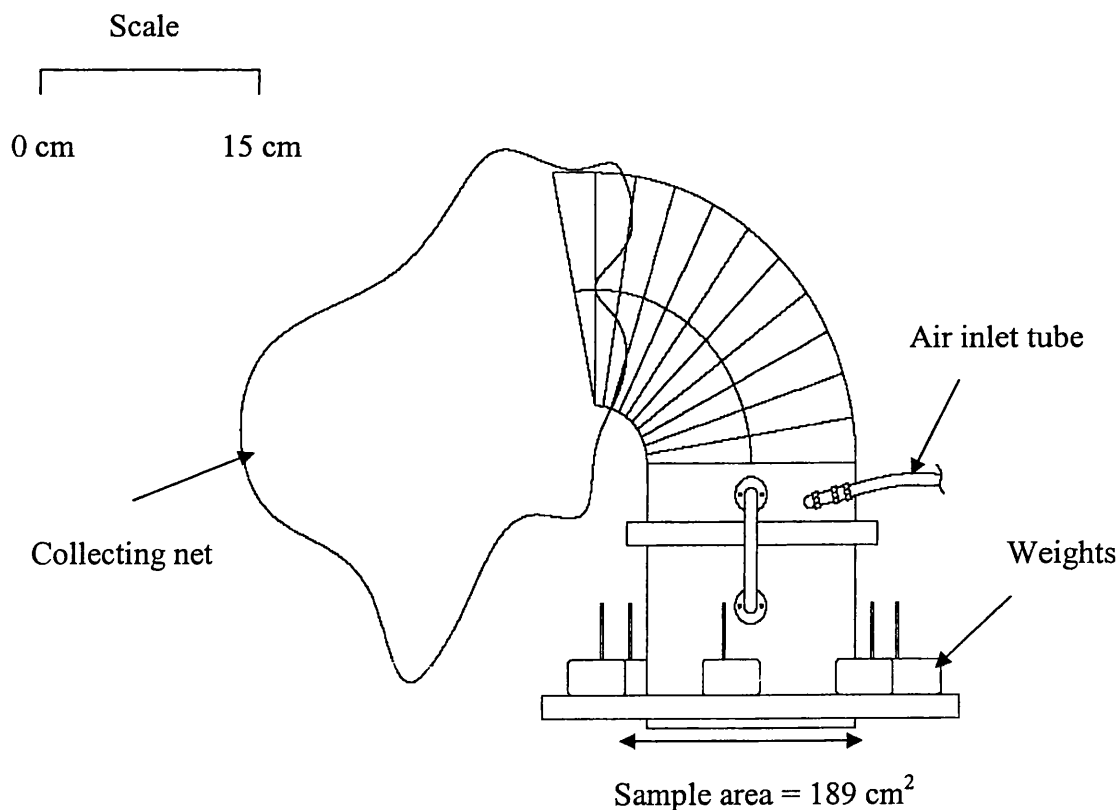


Figure 3.2 A modified airlift sampler.

with any attached invertebrates, was lifted in the air/water emulsion, up through the tube and deposited into a fine mesh (400 μ m) collection net placed over the opening of the tube (Mackey 1972; Southwood 1978). This sampler was designed to operate over a greater range of water depths than most airlift

samplers (Mackey 1972; Pearson et al. 1973). The design of the sampler meant that this device could be deployed at depths of as little as 50cm and yet still function well at depths of 30m. Fifteen samples were collected at each site by administering a 10 second blast of air to disturb the substrate at a constant sampling depth of approximately 2.5 meters. Sampling was conducted at a depth of 2.5 meters at all sampling sites to reduce the confounding effects upon the invertebrate community of changes in depth throughout the littoral zone. In addition, two bulk samples consisting of five, 10-second blast samples were taken from each site to increase the weight of tissue available for analysis. The invertebrates from these additional samples were used purely to provide supplementary biomass for stable isotope analysis and not to determine community structure at the sampling locations.

3.3.2 *Laboratory methods*

Fish

Once removed from the net, fish were identified and fork length and weight measurements taken prior to dissection. A sample of white muscle tissue from all fish caught was taken from an area on the flank, immediately posterior to the operculum. All bone and skin was removed from the tissue sample at this stage. The tissue was then placed in a glass vial and frozen immediately until further preparatory work could be carried out. Freezing and freeze drying have been found to be the only preservation methods that do not affect carbon and nitrogen stable isotope ratios (Bosley and Wainright 1999). For the principal study species, ruffe, additional tissue samples were also taken. The opercular bone

was dissected from the right side and the liver was also removed. These tissues were frozen immediately in glass vials. Stomach contents were removed from ruffe by dissection. Only material present in the main stomach was removed for identification. Stomach contents were stored in 70% ethanol and subsequently identified to the lowest taxonomic level possible. A count was made of the number of prey items in each stomach.

Invertebrates

In the laboratory, invertebrate samples were emptied into plastic trays and hand sorted for a period of 30 minutes or until all invertebrates had been removed from the sample. The invertebrates were then identified to the lowest taxonomic level possible, counted, and frozen for further analysis. Any species which could not be identified (eight), were assigned a letter (A to H) and counted as a species in order to make an assessment of richness. Identification was made using Freshwater Biological Association identification keys and reference specimens from the Harry Slack Memorial Collection located at the University Field Station, Loch Lomond.

3.3.3 Procedure for determination of dry weight measurements

After identification, fish tissue and whole invertebrate samples were transferred into pre-weighed vials. The material was dried at 60°C for at least 36 hours. After cooling in a desiccator, dry mass was determined to the nearest 0.01mg.

3.3.4 *Preparation of opercular bones for lipid extraction*

Opercular bones were dissected from ruffe and any attached tissue removed using a scalpel. About 50mg of bone was placed in a 10ml glass vial for decalcification in order to remove the inorganic mineral phase of the bone. This was carried out so that an accurate mass for the collagen present in the sample could be determined, and also to remove any potential carbon contamination from carbonate preserved within the hydroxyapatite mineral of the bone (DeNiro and Epstein 1978). 5ml of 0.5 N HCl was added to the sample. This was agitated for 10 minutes and then refrigerated for 2-3 days. At the end of this period, to assess whether decalcification was complete, samples were removed from their vials and checked for flexibility. When it was possible to bend or squash samples easily, decalcification was deemed complete. If samples remained rigid, the acid was changed and the samples refrigerated for a further 2 days. Once decalcification was complete, the HCl was removed and the samples washed 5 times with distilled water, then oven dried at 60°C to constant weight in preparation for lipid extraction.

3.3.5 *Preparation of samples for stable isotope analysis*

All samples were dried and ground prior to stable isotope analysis. Fish muscle, liver and bone tissue samples were further treated to remove lipid. Due to small biomass levels it was not possible to remove lipid from invertebrate samples. Differences in the carbon isotopic composition of the major biochemical components of an organism have been identified by DeNiro and Epstein (1977). The lipid fraction is relatively depleted in ^{13}C with respect to the other

components such as protein and carbohydrate and also with respect to the total organism. The observed ^{13}C depletion has been attributed to the process of oxidation of pyruvate to acetyl coenzyme A during the metabolism of glucose (DeNiro & Epstein 1977). Kinetic isotope effects during the pyruvate dehydrogenase reaction account for the ^{13}C depletion of the lipid fraction observed in organisms as they exist in nature. As the basis of lipid synthesis is the same in all organisms, this depletion in ^{13}C may affect ecological interpretations in most species.

Lipids were removed using the following method. Six ml of methanol: chloroform: water in the ratio 2:1:0.8 was added to the finely ground sample and agitated. The mixture was then spun down at 1800g in a centrifuge and 2 ml of water was added to the vial. Upon the addition of water, the solution separated into two distinct phases: an upper layer of methanol and water and a bottom layer of chloroform which contained the lipids. A large amount of lipid in the chloroform phase gave a milky appearance. The supernatant was decanted from the sample and discarded. The procedure was repeated until the supernatant remained clear when the water was added, indicating that all lipids had been successfully removed. In some cases it was necessary to repeat this step up to four times to achieve complete removal of lipids. Once all lipids had been removed, and the supernatant remained clear when water was added, the sample was washed 5 times with water and the pellet of lipid-free tissue was dried at 60°C to constant weight. These lipid-free samples were reground prior to analysis.

3.3.6 *Stable isotope analysis of samples*

Approximately 1 mg of tissue from each sample was loaded into a 4x6 mm tin capsule and combusted in a Carlo Erba C/N/S analyser (Thermoquest, Hemer Hempsted, UK) interfaced with a Finnigan Tracer Matt continuous flow isotope ratio mass spectrometer (CF-IRMS). All stable isotope ratios are reported in permil (‰) using the δ notation according to the following equation:

$$\delta X = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

Where X is ^{13}C or ^{15}N and R is the corresponding ratio of $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. R_{standard} for $\delta^{13}\text{C}$ is the Pee Dee Belemnite and for $\delta^{15}\text{N}$ is atmospheric nitrogen (Smith et al. 1996; Ponsard and Arditi 2000). The precision with which $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ can be measured is at least $\pm 0.3\text{‰}$, and previous work using the same machine has shown that replicate analysis of samples from fish, results in a very high degree of sample reproducibility (McCarthy and Waldron 2000).

Stable isotope analysis was carried out on white muscle, liver and opercular bone tissue of ruffe collected from all six sampling sites during the summer sampling period. Stable isotope analysis was also carried out on invertebrates collected from all six sampling sites within Loch Lomond (Appendix 2).

3.3.7 *Baseline correction of isotope signatures*

Due to the differences in the isotopic ratios of carbon $\delta^{13}\text{C}$ and nitrogen $\delta^{15}\text{N}$ available for uptake by organisms at the base of the food web, $\delta^{15}\text{N}_{\text{base}}$ and $\delta^{13}\text{C}_{\text{base}}$ have high spatial and temporal variation between, and within, aquatic

systems (Toda and Wada 1990; Kling et al. 1992; Kline et al. 1993; Gu et al. 1994; Cabana and Rasmussen 1996). Without suitable estimates of baseline $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values at each site, there is no way to determine if variation in lipid-free stable isotope signatures reflect changes in food web structure and carbon flow, or just variation in baseline isotopic signatures. Variation in $\delta^{15}\text{N}_{\text{base}}$ and $\delta^{13}\text{C}_{\text{base}}$ is difficult to resolve using plankton because of the large temporal variability in isotopic signatures of small organisms that have fast tissue turnover (Cabana and Rasmussen 1996; Vander Zanden et al. 1997). Snail isotopic signatures at each of the six sampling sites were used in this instance to represent baseline values, as snail isotopic signatures have been shown to be a reliable indicator of baseline isotopic signature (Vander Zanden et al. 1999; Post 2002). Baseline correction of stable isotope values was carried out according to Vander Zanden et al (1997) using the following formula:

$$\text{Trophic position} = [(\text{organism } \delta^{15}\text{N} - \text{baseline } \delta^{15}\text{N})/3.4] + 2$$

Where 3.4 represents a 1.0 trophic level increment in $\delta^{15}\text{N}$.

3.4 Statistical Analysis

The degree of species diversity observed in the communities at each sampling site was determined using species richness scores, expressed simply as the number of species recorded at each sampling site (Stiling 2002). One major problem with this approach is that it does not take species abundance into account. To overcome this, the Shannon Weiner index was also calculated using the following equation.

$$H_s = - \sum p_i \ln p_i$$

Where p_i is the proportion of individuals found in the i th species and \ln is the natural logarithm (Stiling 2002). The Shannon-Weiner index has an advantage over simple species richness scores in that it integrates an element of species abundance in the calculation of species diversity (Stiling 2002). The Shannon-Weiner index was also calculated for the stomach contents of fish at each site to give an indication of feeding niche width between sites. t -Tests ($p > 0.05$) were employed to identify seasonal differences. Two way ANOVA was used to investigate differences in stable isotope signature between tissue types and sites.

3.5 Results

3.5.1 *Are there differences in community structure between study sites?*

Species richness and Shannon-Weiner scores were calculated for the combined fish and invertebrate communities at each of the six sampling sites in Loch Lomond (Table 3.3).

Figure 3.3 illustrates the species richness scores for each of the six sampling sites (winter and summer data combined). When combined community structure was explored over both sampling periods, the northern (Inverhoulin and Rubha Mor sites) and southern (Inchlonaig and Ross Priory sites) basins displayed similarities in terms of species richness. The two mid basin sampling sites (Ross Point and Sallochay Bay) produced the most extreme results. Despite the fact that these sites are geographically closer than the others, they lay at opposite ends of

Table 3.3 Whole community diversity scores for sampling sites in Loch Lomond.

	Species Richness Score	Shannon-Weiner Score
Inverhoulin	27	2.28
Rubha Mor	28	2.64
Ross Point	23	1.91
Salloch Bay	32	2.72
Inchlonaig	23	2.66
Ross Priory	28	2.37

the diversity scale. Salloch Bay in the mid basin of Loch Lomond had the highest diversity recorded during sampling with a score of 32 while Ross Point, also located in the mid basin of the loch, had the lowest number of taxa with only 23 recorded. Inverhoulin, the most northerly of all the sampling sites, and Ross Priory, the most southerly sampling site, had the same species richness score of 28 (Figure 3.3).

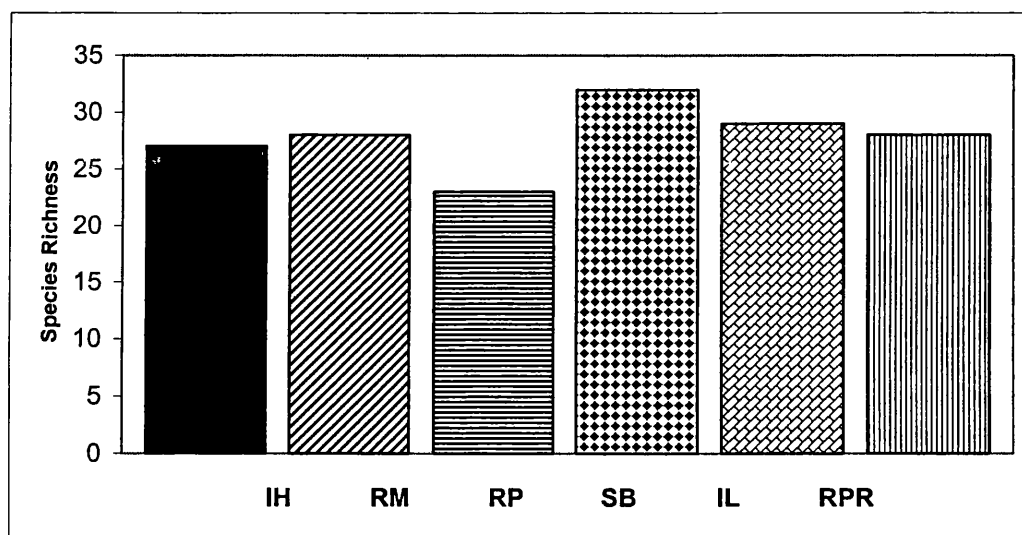


Figure 3.3 Loch Lomond whole (fish and invertebrate) community structure expressed as species richness. Winter and summer data combined. Sites are ordered from north to south, Inverhoulin (IH), Rubha Mor (RM), Ross Point (RP), Salloch Bay (SB), Inchlonaig (IL) and Ross Priory (RPR).

To incorporate an element of evenness into the exploration of whole community structure, the Shannon-Weiner Index was calculated. Data from both winter and summer sampling periods were combined to incorporate temporal changes which may occur due to seasonality (Figure 3.4).

The Shannon-Weiner scores display the same pattern as those of species richness. The sampling locations in the mid basin of the loch, Ross Point and Salloch Bay, had the lowest and highest scores respectively. There was no evidence of a predictable pattern of community structure emerging with the transition from the north to the south basin of Loch Lomond, despite the general trend of increasing pH, BOD and phosphate level at the more southerly sampling sites (Table. 3.1).

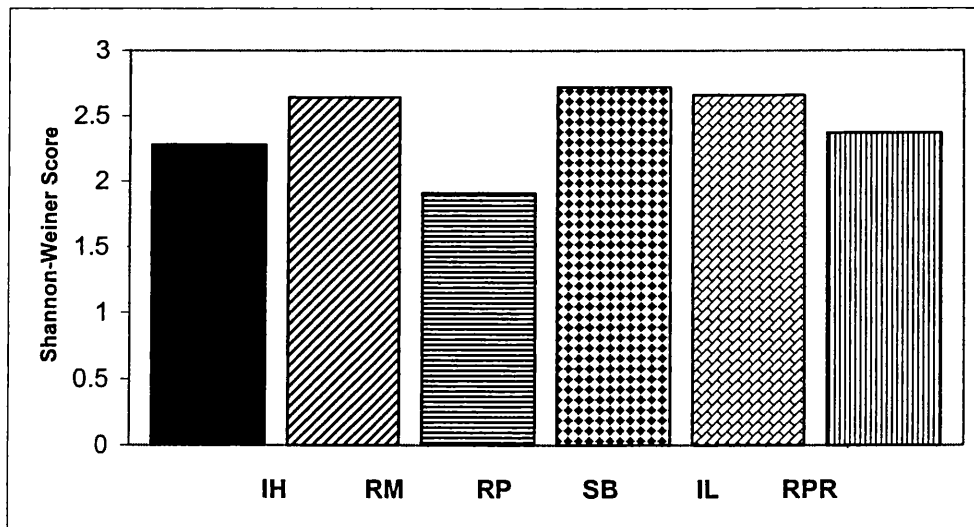


Figure 3.4 Loch Lomond whole (fish and invertebrate) community structure expressed as a Shannon-Weiner score. Winter and summer data combined. Sites are ordered from north to south, Inverhoulin (IH), Rubha Mor (RM), Ross Point (RP), Salloch Bay (SB), Inchlonaig (IL) and Ross Priory (RPR)

To examine further the structure of the fish and invertebrate communities at each of the sampling sites, samples collected during the winter and the summer sampling periods were examined separately. As may be expected, species richness scores were significantly higher ($t = -19.295$, $P = 0.001$) for invertebrate communities examined than for fish communities (Figure 3.5). Once again the major difference in species richness scores lay between the mid basin sites of Salloch Bay and Ross Point. However, the most northerly of the sampling sites, Inverhoulin, displayed the lowest fish species richness score.

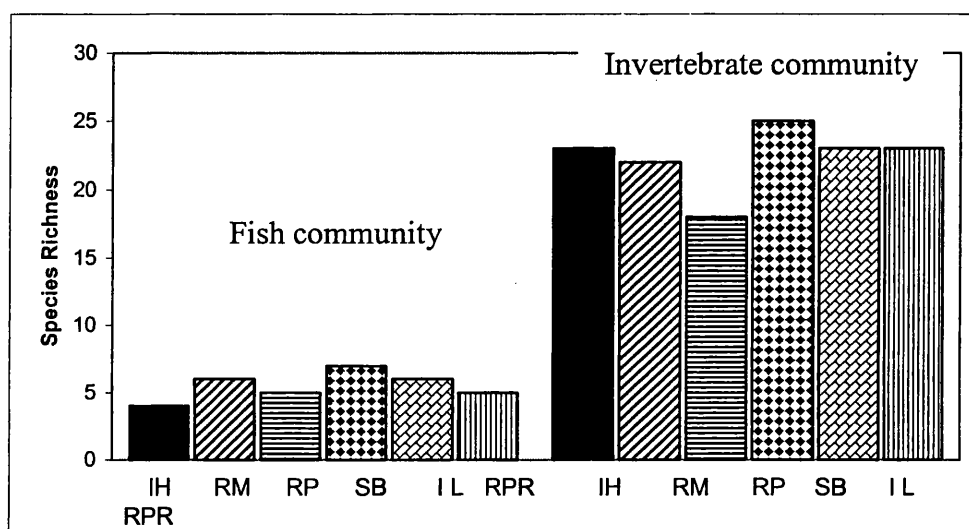


Figure 3.5 Fish and invertebrate species richness for combined winter and summer data. Sites are ordered from north to south, Inverhoulin (IH), Rubha Mor (RM), Ross Point (RP), Salloch Bay (SB), Inchlonaig (IL) and Ross Priory (RPR).

Shannon-Weiner scores were also calculated separately for the fish and invertebrate communities at each of the sampling sites. Once again, Salloch

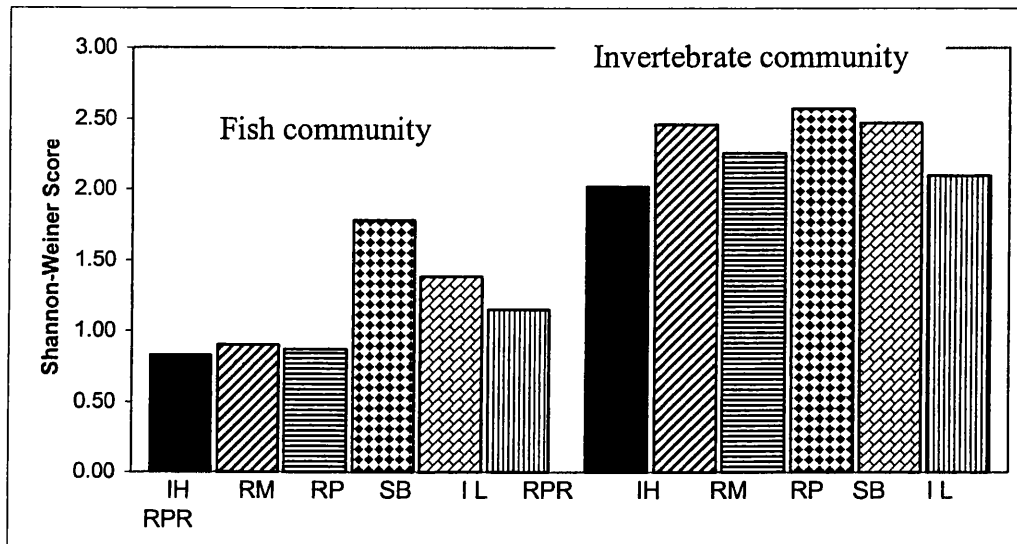


Figure 3.6 Fish and invertebrate Shannon-Weiner scores for combined winter and summer data. Sites are ordered from north to south, Inverhoulin (IH), Rubha Mor (RM), Ross Point (RP), Salloch Bay (SB), Inchlonaig (IL) and Ross Priory (RPR).

Bay displayed the highest Shannon-Weiner score for both fish and invertebrate communities while Inverhoulin had the lowest score (Figure 3.6).

Fish catches were dominated by large numbers of perch (*Perca fluviatilis*) at all sites except for Salloch Bay where ruffe made up 45% of the catch and was the most common species caught in gill nets. Table 3.4 details catch per unit effort data for fish collected from the six sampling sites on a seasonal basis. The pattern of dominance in catch of perch at sampling sites is reflected in the Shannon-Weiner scores (Figure 3.6). The three more northerly sites, Inverhoulin,

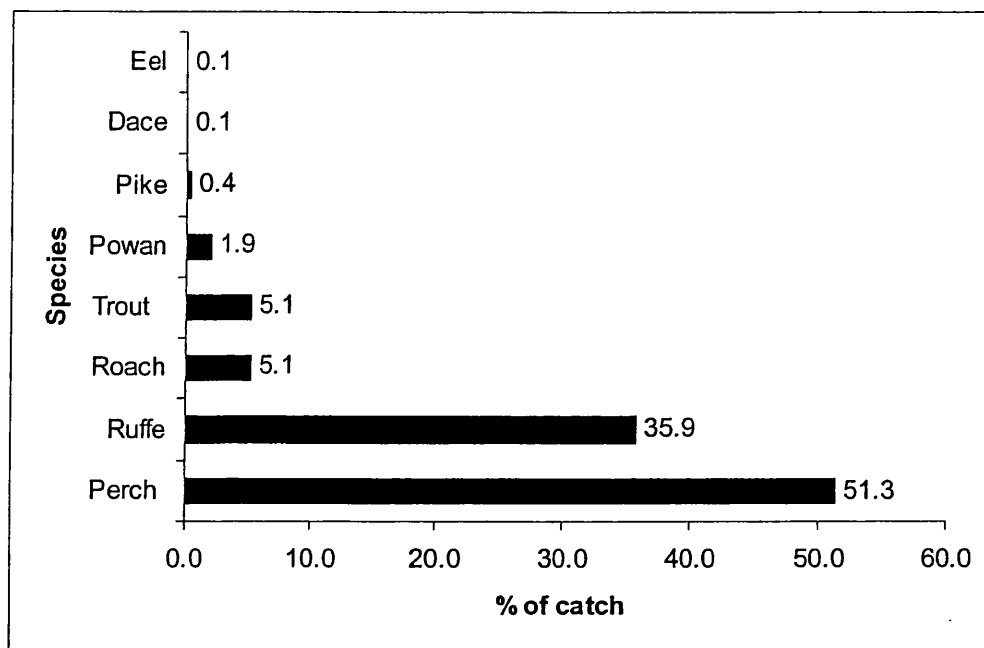


Figure 3.7 Fish catches in Loch Lomond reported as the % of all fish caught.

Rubha Mor and Ross Point, where perch and ruffe made up more than 92% of the catches, had low Shannon-Weiner scores. Perch and ruffe also dominated the southern basin sites of Inchlonaig and Ross Priory but the percentage of these species caught in gill nets was lower at 72% and 76% respectively. Figure 3.7 illustrates the total percentage of fish caught over the two sampling seasons

at all sites. Where fish catches were examined on a seasonal basis the number of fish caught at each of the sampling sites was lower in winter than in summer in (Table 3.4).

Table 3.4 Catch Per Unit Effort data for fish collected from six sampling sites in Loch Lomond.

	Inverhoulin		Rubha Mor		Ross Point		Salloch Bay		Ross Priory		Inchlonaig		TOTAL
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	
Dace	0	0	0	0	0	0	2	0	0	0	0	0	2
Eel	0	0	0	0	0	0	0	0	2	2	0	0	2
Perch	4	139	0	186	0	219	4	18	0	163	0	36	769
Pike	0	0	0	2	0	0	2	0	0	0	2	0	6
Powan	0	2	0	2	0	11	2	10	0	0	0	2	29
Roach	0	0	0	4	0	3	2	0	0	62	0	6	77
Ruffe	16	32	5	56	50	257	22	23	17	50	2	8	538
Trout	10	3	16	0	10	0	20	0	5	5	6	2	77
TOTAL	30	176	21	250	60	490	54	51	22	282	10	54	1500

3.5.2 Are there seasonal differences in community structure within sites?

Species richness scores were calculated separately for fish and invertebrates in summer and winter. A paired *t*-test was performed on this data to establish if site community structure changed significantly with season.

The paired *t*-test demonstrated that there was no significant difference in fish species richness scores between summer and winter ($t = -0.52$, $P = 0.632$).

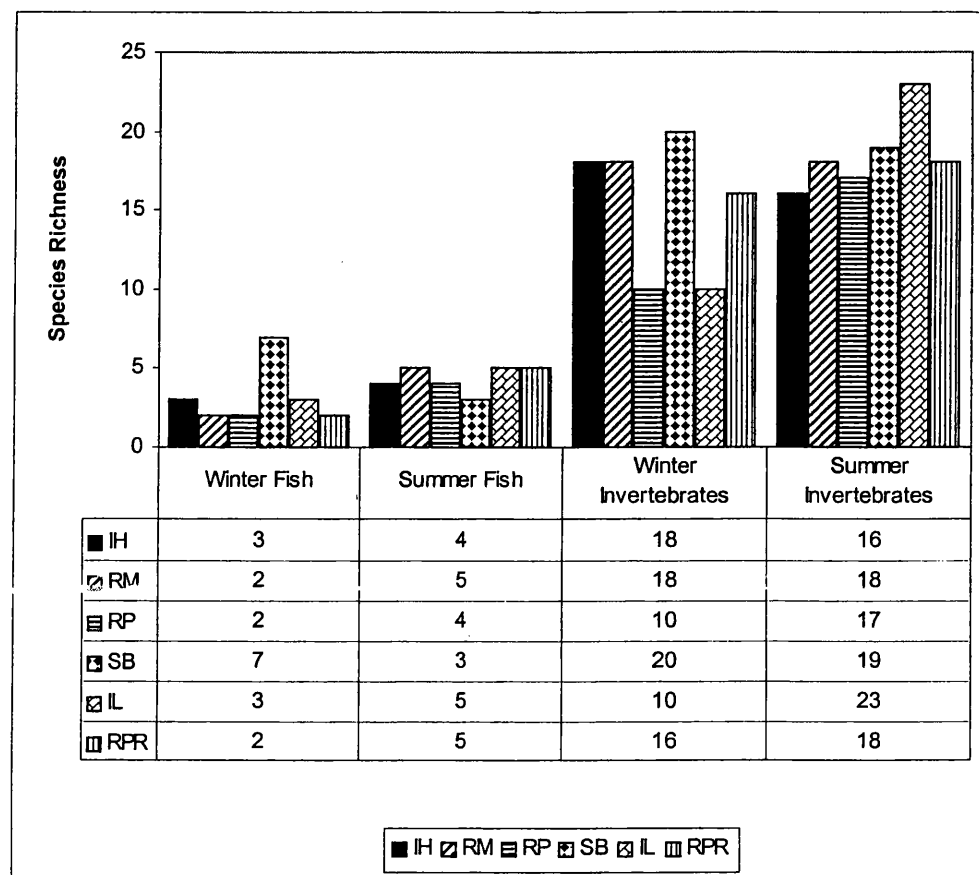


Figure 3.8 Species richness for fish and invertebrate communities in Loch Lomond sampled in winter (03/02) and summer (09/02). Sites are ordered from north to south, Inverhoulin (IH), Rubha Mor (RM), Ross Point (RP), Salloch Bay (SB), Inchlonaig (IL) and Ross Priory (RPR)

Neither was there any clear pattern of change evident in invertebrate species richness between the two sampling periods ($t = -0.50$, $P = 0.636$).

In winter, Salloch Bay fish and invertebrate species richness scores were higher than those for all other sampling sites. During the summer sampling period, the fish community species richness between sites was much more uniform, with a difference of only 2 between the minimum and maximum scores compared to that of the winter period where there was a difference of 5 (Figure 3.8). The same trend was observed for the difference in minimum and maximum species richness scores of the invertebrate communities between sites. This decreased from 10 in winter to 8 in the summer sampling periods (Figure 3.8).

As was the case with species richness scores, paired t -tests revealed no significant differences in Shannon-Weiner scores between sites in the two sampling periods for invertebrates ($t = -1.34$, $P = 0.23$) or fish ($t = -1.08$, $P=0.328$). Overall, there seems to be no clear differences in structure except for fish in summer where the Shannon-Weiner score apparently increased from the north to the south basin of Loch Lomond. A two-sample t test was carried out on this summer data and demonstrated that the increase in Shannon-Weiner scores from the north to the south basin of Loch Lomond was significant ($t=6.07$, $P=0.02$).

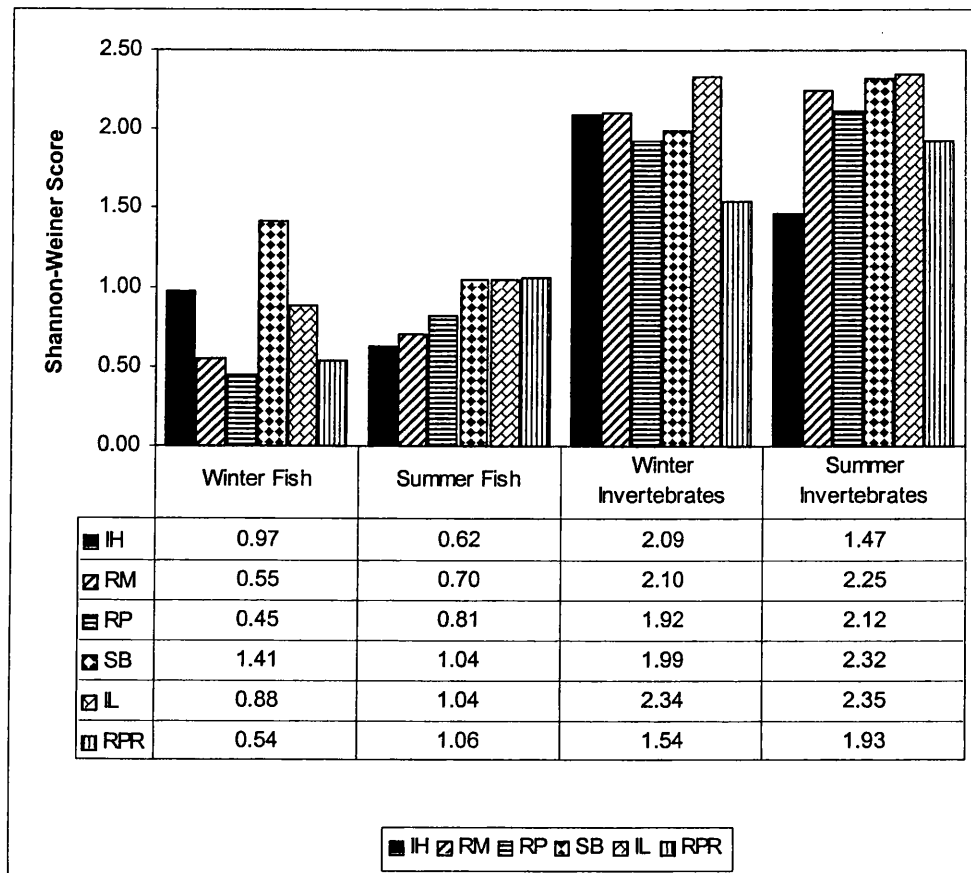


Figure 3.9 Shannon-Weiner Scores for fish and invertebrate communities in Loch Lomond sampled on a seasonal basis. Sites are ordered from north to south, Inverhoulin (IH), Rubha Mor (RM), Ross Point (RP), Salloch Bay (SB), Inchlonaig (IL) and Ross Priory (RPR).

3.5.3 Stomach Contents

To determine variability in diet choice, ruffe stomach contents were removed and identified (Table 3.6). Species richness (Table 3.5) and Shannon-Weiner scores (Figure 3.10 and Figure 3.11) were calculated in order to provide an indication as to the diversity and evenness of species ingested by ruffe at each of the six study sites. This was carried out for individuals collected from each site during the summer and winter sampling periods.

Table 3.5 Species richness scores of ruffe stomach contents

	Ruffe Stomach Contents Species Richness		
	Winter	Summer	Combined
Inverhoulin	3	10	11
Rubha Mor	6	12	12
Ross Point	2	4	5
Sallochay Bay	6	12	12
Inchlonaig	4	7	11
Ross Priory	5	9	8

There was a significant difference in species richness scores of ruffe stomach contents between winter and summer (paired $t = 5.81$, $P = 0.002$). In all cases the number of species present in the stomach contents was higher in summer than in winter. Table 3.6 provides details on ruffe stomach contents from each of the sampling sites during both the winter and summer sampling periods.

Gammaridae and chironomidae larvae were present in stomach contents of ruffe from all sites collected during the summer sampling period however there was no common species recorded in stomach contents during the winter sampling period. The stomach contents of ruffe caught at the sampling site at Ross Point had the lowest species richness in both winter (2) and summer (4) while fish from Sallochay Bay and Rubha Mor had the highest stomach content species richness scores in winter (6) and in summer (12) (Table 3.5).

Table 3.6 Ruffe stomach contents for each of the sampling sites in winter and summer.

	Inverhoulin		Rubha Mor		Ross Point		Salloch Bay		Inchlonaig		Ross Priory	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
Cladocera	x	✓	✓	✓	x	x	✓	✓	x	✓	x	✓
Copepoda	x	x	x	x	x	x	x	x	x	✓	x	x
Asellidae	✓	✓	✓	✓	x	x	✓	✓	✓	x	✓	✓
Sphaeriidae	x	✓	✓	✓	x	x	✓	✓	✓	✓	x	✓
Physidae	x	x	x	x	x	x	x	✓	x	x	x	✓
Coleoptera pupa	x	✓	x	✓	x	x	x	x	x	x	x	✓
Coleoptera larvae	x	x	x	✓	x	x	x	x	x	x	x	x
Gammaridae	✓	✓	x	✓	✓	✓	x	✓	x	✓	✓	✓
Ceratopogonidae	✓	x	✓	✓	x	x	✓	✓	x	✓	x	✓
Chironomidae Larvae	x	✓	✓	✓	x	✓	✓	✓	✓	✓	x	✓
Chironomid pupa	x	✓	x	✓	x	✓	✓	✓	✓	✓	✓	x
Ephemeroptera	x	✓	x	x	x	x	x	x	x	x	✓	x
Plecoptera	x	✓	x	✓	x	✓	x	✓	x	x	✓	x
Trichoptera	x	✓	✓	✓	x	x	x	✓	x	x	x	x
Hemiptera	x	x	x	✓	x	x	x	x	x	x	x	x
Eggs	x	x	x	x	✓	x	x	✓	x	x	x	x
Hydracarina	x	x	x	x	x	x	x	✓	x	x	x	✓

Ruffe stomach content data was also expressed as Shannon-Weiner scores. There were no significant difference between sites in mean Shannon-Weiner values of ruffe stomach contents collected in winter ($F_{5,64} = 0.797$, $P = 0.556$). Rubha Mor fish had the highest stomach content Shannon-Weiner value of 0.39 while Ross Priory had the lowest value of 0.13 (Figure 3.10).

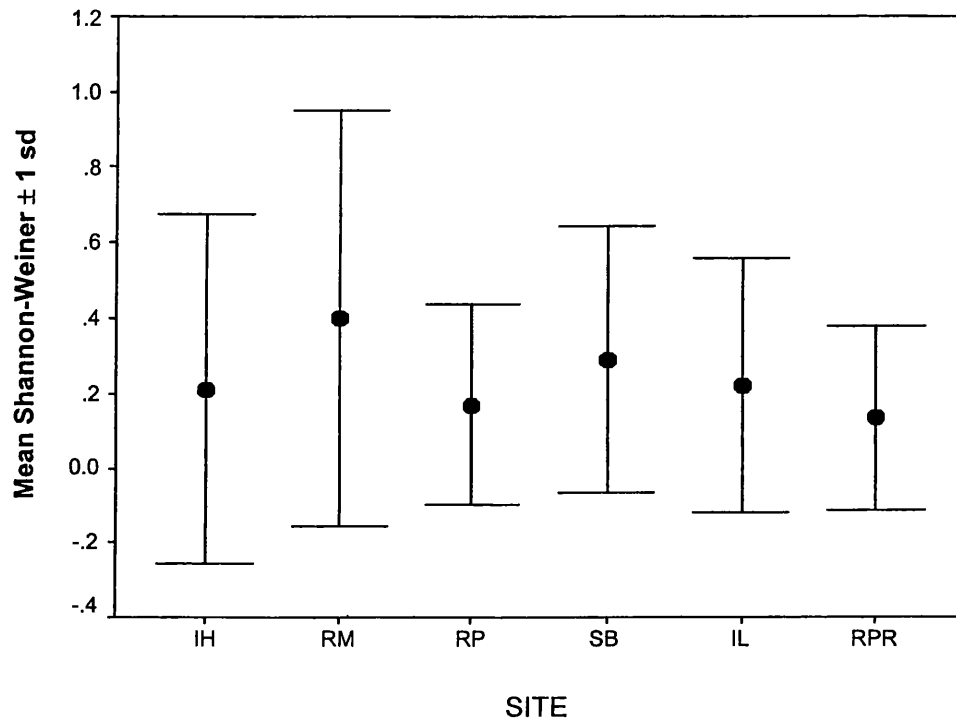


Figure 3.10 Shannon-Weiner scores (scores \pm 1 standard deviation) for stomach contents of ruffe collected during the winter sampling period. Sites are ordered from north to south, Inverhoulin (IH), Rubha Mor (RM), Ross Point (RP), Salloch Bay (SB), Inchlonaig (IL) and Ross Priory (RPR).

However, there were significant differences in stomach content Shannon-Weiner values between the six sampling sites sampled in summer ($F_{5,79} = 5.016$, $P < 0.001$). Multiple comparison tests demonstrated that Ross Point was significantly different from Salloch Bay ($p = 0.007$), Inchlonaig ($p = 0.001$) and from Ross Priory ($p = 0.009$). Inchlonaig had the highest stomach contents diversity score while Ross Point had the lowest score and lowest variance (Figure 3.11).

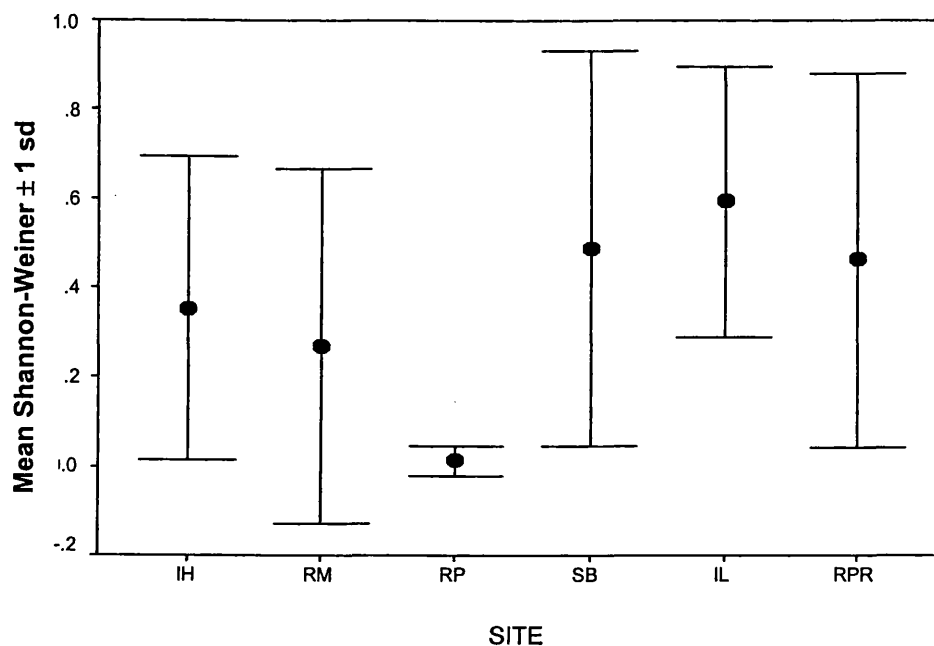


Figure 3.11 Shannon-Weiner scores (± 1 standard deviation) for stomach contents of ruffe collected during the summer sampling period. Sites are ordered from north to south, Inverhoulin (IH), Rubha Mor (RM), Ross Point (RP), Salloch Bay (SB), Inchlonaig (IL) and Ross Priory (RPR)

3.5.4 *Stable Isotope Analysis*

Are there seasonal differences in isotopic signature between sites?

White muscle tissue was dissected from ruffe collected at all six sampling sites in both the winter and summer sampling periods and analysed to determine if there was any shift in the isotopic signature of this tissue with changing site and season. All stable isotope results and values quoted have been baseline corrected.

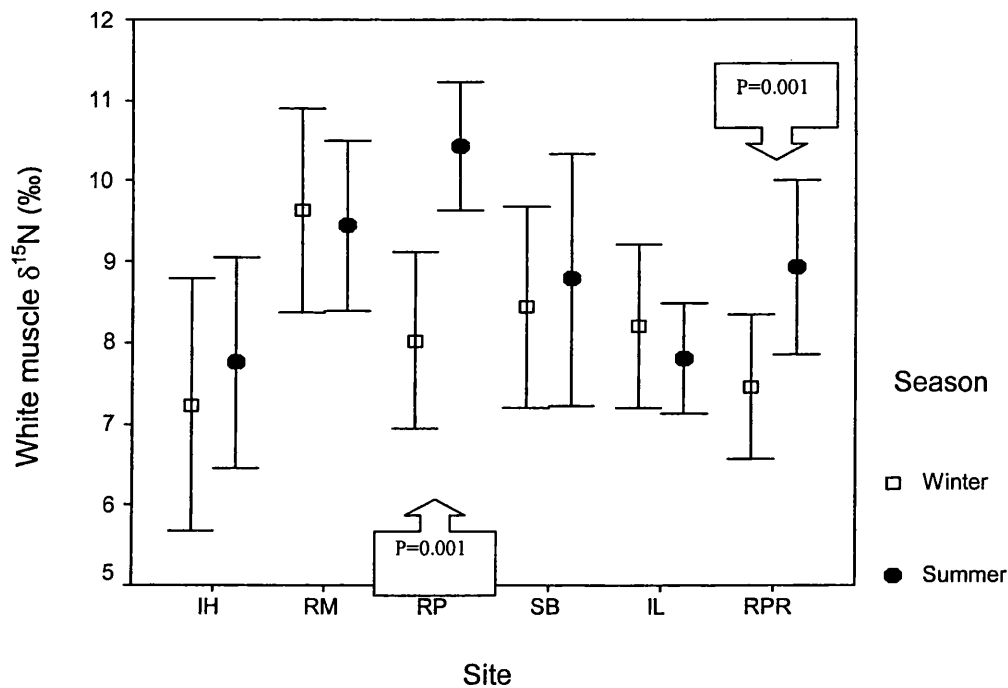


Figure 3.12 Mean $\delta^{15}\text{N}$ stable isotope signature of ruffe muscle tissue ± 1 standard deviation illustrating seasonal differences within sites. Sites are ordered from north to south, Inverhoulin (IH), Rubha Mor (RM), Ross Point (RP), Salloch Bay (SB), Inchlonaig (IL) and Ross Priory (RPR) for winter and summer data. An arrow box containing a probability value marks significant differences in stable isotope signature between summer and winter within a single site. All quoted isotope values are baseline corrected.

Figure 3.12 illustrates that there was a significant difference between sites in the mean $\delta^{15}\text{N}$ stable isotope signature of ruffe white muscle tissue recorded in winter ($F_{5,72} = 6.185$, $P < 0.01$) and summer ($F_{5,84} = 12.496$, $P = 0.01$). This suggests that ruffe in different sites are feeding on isotopically distinct prey items. When all sampling sites were considered together, there was also a significant difference in ruffe muscle $\delta^{15}\text{N}$ signatures between the two sampling periods, winter and summer ($t_{166} = -3.348$, $p = 0.01$) suggesting that changes in dietary preferences take place on a seasonal basis. Mean $\delta^{15}\text{N}$ values decreased very slightly but not significantly between winter and summer at Rubha Mor in the north basin, and at Inchlonaig in the south basin of the loch. Fish tissues at all other

sampling sites were enriched in ^{15}N between sampling periods and this was statistically significant for Ross Point ($t = -36.012$, $p = 0.001$) where there was no overlap in the standard deviation of muscle $\delta^{15}\text{N}$ between seasons. This was also the case at Ross Priory ($t = -36.380$, $p = 0.001$), suggesting that there was a dramatic shift in dietary preferences at these sites between seasons (Figure 3.12).

Ruffe white muscle tissue values exhibited a significant difference in mean $\delta^{13}\text{C}$ values between sites in summer ($F_{5,84} = 7.906$, $P < 0.01$) and winter ($F_{5,72} = 2.775$, $P = 0.024$) (Figure 3.13). However there was no significant difference in

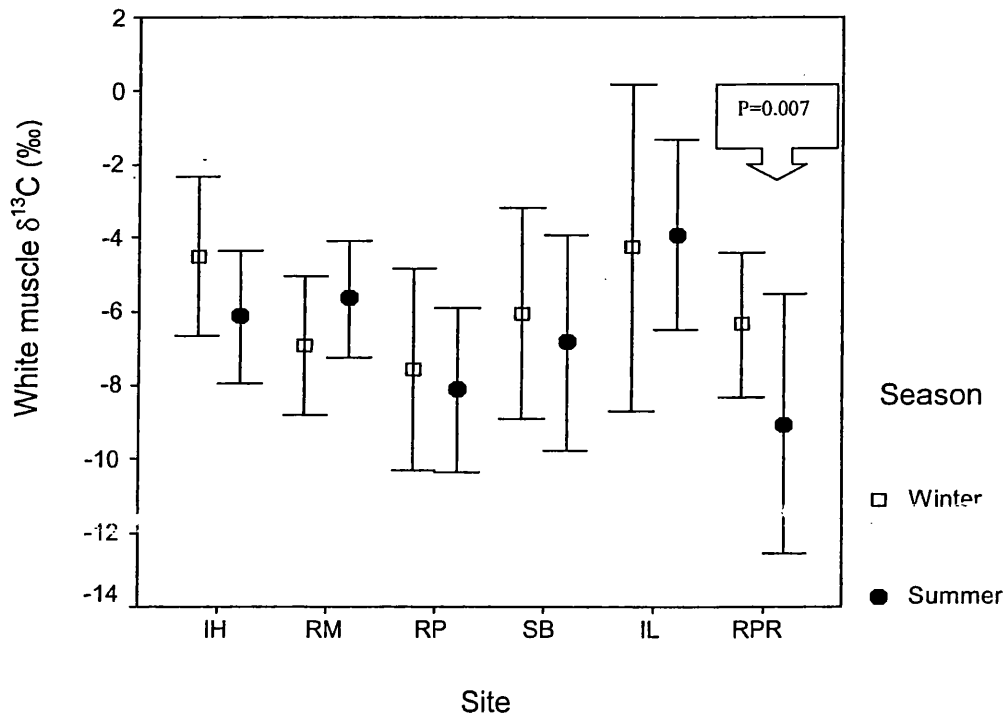
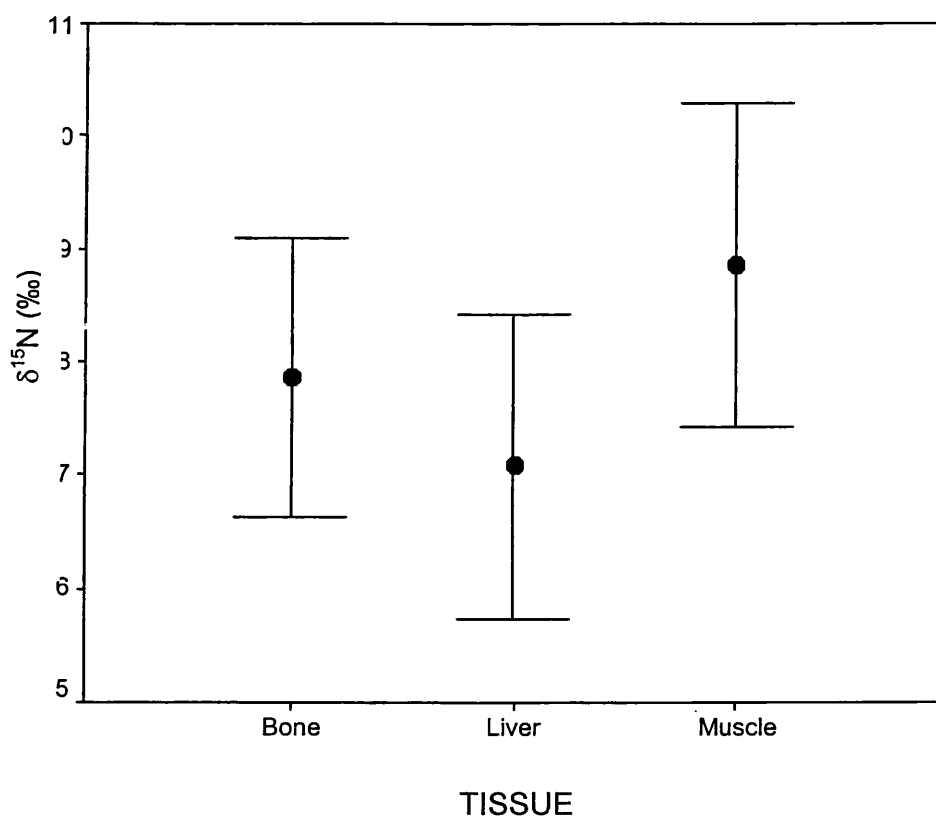


Figure 3.13 $\delta^{13}\text{C}$ stable isotope signature of ruffe muscle tissue (mean \pm 1 standard deviation) for summer and winter sampling periods. Sites are ordered from north to south, Inverhoulin (IH), Rubha Mor (RM), Ross Point (RP), Salloch Bay (SB), Inchlonaig (IL) and Ross Priory (RPR) for winter and summer data. An arrow box containing probability value marks significant differences in stable isotope signature between summer and winter.

seasonal $\delta^{13}\text{C}$ signatures ($t_{166} = 1.366$, $p = 0.174$) suggesting that carbon signatures and sources remain relatively constant within Loch Lomond over time (Figure 3.13).

Variation in isotopic signature between tissues

A One-Way ANOVA was carried out to explore the differences in isotopic signature of the three ruffe tissue types (white muscle, liver and bone). There were highly significant differences in the $\delta^{15}\text{N}$ isotopic signatures of these three tissues ($F_{2, 267} = 40.042$, $P < 0.001$) and a multiple comparison test revealed that differences in isotope signature were highly significant between muscle and liver, ($t = 1.7818$, $P = 0.001$), muscle and bone ($t = 1.0056$, $P = 0.001$) and liver and bone ($t = -0.7762$, $P = 0.001$) (Figure 3.14). This is most likely due to the differing



tissue turnover rates, which in turn reflect the dietary preferences of ruffe as they change seasonally and as a result of increasing body size.

There were also highly significant differences in the mean $\delta^{15}\text{N}$ signatures of muscle ($F_{5,84} = 12.496$, $P < 0.001$), liver ($F_{5,84} = 7.237$, $P < 0.001$) and bone ($F_{5,84} = 8.714$, $P < 0.001$) tissues between sites (Figure 3.15). Once again a multiple comparison test was carried out to identify differences in isotopic signature of muscle tissue (Table 3.3), liver (Table 3.4) and bone (Table 3.5) between sites.

Table 3.7 Multiple comparison of muscle $\delta^{15}\text{N}$ signatures between sites. P values are provided for sites which were significantly different.

	Inverhoulin	Rubha Mor	Ross Point
Inverhoulin	-	-	-
Rubha Mor	0.001	-	-
Ross Point	0.001		-
Sallochy Bay			0.002
Inchlonaig		0.001	0.001
Ross Priory			0.005

Table 3.8 Multiple comparison of liver $\delta^{15}\text{N}$ signatures between sites. P values are provided for sites which were significantly different.

	Inverhoulin	Rubha Mor	Ross Point
Inverhoulin	-	-	-
Rubha Mor	0.001	-	-
Ross Point	0.001		-
Sallochy Bay			0.008
Inchlonaig		0.041	0.006
Ross Priory			

Table 3.9 Multiple comparison of bone $\delta^{15}\text{N}$ signatures between sites. P values are provided for sites which were significantly different.

	Inverhoulin	Rubha Mor	Ross Point
Inverhoulin	-	-	-
Rubha Mor	0.040	-	-
Ross Point	0.001	0.010	-
Salloch Bay	0.028		0.015
Inchlonaig			0.001
Ross Priory	0.040		0.010

Ruffe white muscle tissue was consistently enriched in ^{15}N relative to bone and liver tissue from the same site. Liver stable isotope signatures, which reflect recent diet, are generally less enriched in $\delta^{15}\text{N}$ while bone signatures, which integrate dietary preferences over a longer time scale, tend to show intermediate enrichment. This suggests that dietary preferences have altered temporally at each site and this change is reflected in the stable isotope signature of muscle and liver. Bone isotopic signatures were intermediate between muscle and liver signatures and from this it would seem that bone isotopic signatures reflect overall change as would be expected from a tissue capable of long term integration.

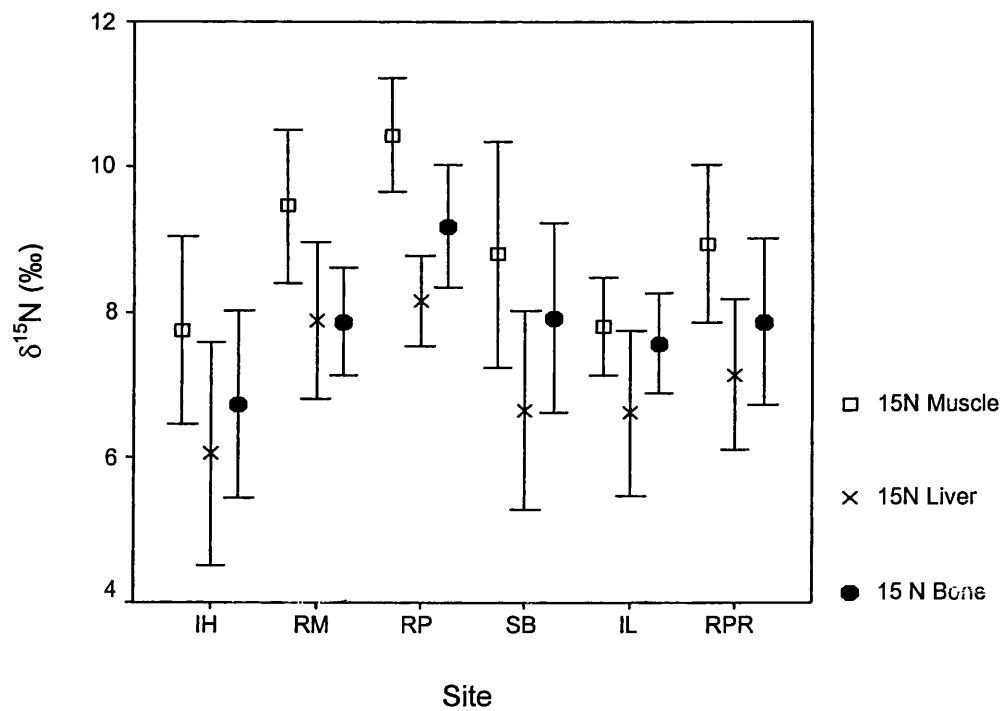


Figure 3.15 Mean $\delta^{15}\text{N} \pm 1$ standard deviation of muscle, liver and bone samples collected from ruffe at each of the sampling sites. Sites are ordered from north to south, Inverhoulin (IH), Rubha Mor (RM), Ross Point (RP), Salloch Bay (SB), Inchlonaig (IL) and Ross Priory (RPR).

There were also changes in the variance associated with tissue type at the same site. The standard deviation of liver, which has a short turnover time, is relatively high compared to that of muscle and bone at the same site with the exception of Ross Point. This suggests that there have been recent changes in the range of prey taken. This change in feeding niche width (Bearhop et al. 2004) is also accompanied by a reduction in mean $\delta^{15}\text{N}$ values at this site.

A One-Way ANOVA, carried out to explore the differences in $\delta^{13}\text{C}$ of the three ruffe tissue types (white muscle, liver and bone) was also highly significant ($F_{2, 267} = 25.610$, $P = 0.001$) (Figure 3.16). Once again this is most likely a function of the differing tissue turnover rates, which will reflect temporal changes in ruffe dietary

preferences or may possibly reflect a seasonal change in baseline $\delta^{13}\text{C}$ signatures.

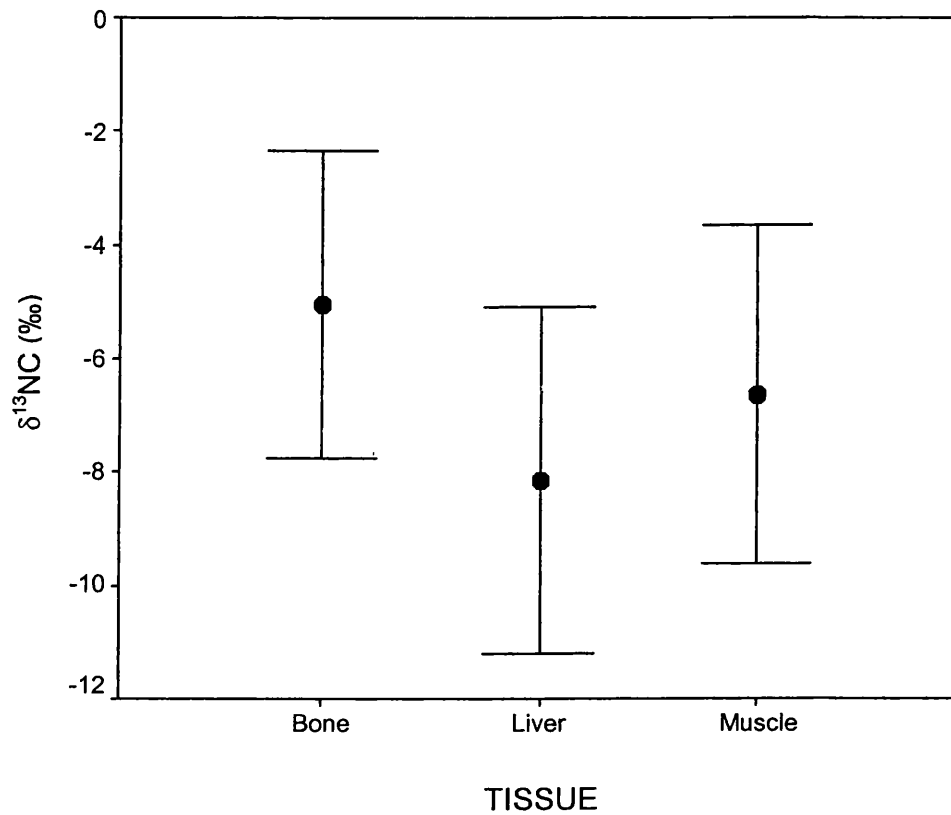


Figure 3.16 Mean bone, liver and muscle tissue $\delta^{13}\text{C}$ and ± 1 standard deviation for all summer sites combined.

Mean $\delta^{13}\text{C}$ signatures also displayed highly significant differences in the signature of muscle ($F_{5,84} = 7.906$, $P < 0.001$), liver ($F_{5,84} = 5.988$, $P < 0.001$) and bone ($F_{5,84} = 8.102$, $P < 0.001$) tissues between sites (Figure 3.17). A multiple comparison test was carried out to identify differences in isotopic signature of muscle tissue (Table 3.3), liver (Table 3.4) and bone (Table 3.5) between sites.

Table 3.10 Multiple comparison of muscle $\delta^{13}\text{C}$ signatures between sites. p values are provided for sites which were significantly different.

	Inverhoulin	Rubha Mor	Ross Point	Sallochy Bay	Inchlonaig	Ross Priory
Inchlonaig			0.001	0.024	-	-
Ross Priory	0.026	0.005			0.001	-

Table 3.11 Multiple comparison of liver $\delta^{13}\text{C}$ signatures between sites. p values are provided for sites which were significantly different.

	Inverhoulin	Rubha Mor	Ross Point	Sallochy Bay	Inchlonaig	Ross Priory
Inchlonaig			0.004		-	-
Ross Priory	0.042	0.021			0.001	-

Table 3.12 Multiple comparison bone of $\delta^{13}\text{C}$ signatures between sites. p values are provided for sites which were significantly different.

	Inverhoulin	Rubha Mor	Ross Point	Sallochy Bay	Inchlonaig	Ross Priory
Ross Point		0.003	-	-	-	-
Sallochy Bay		0.034		-	-	-
Inchlonaig					-	-
Ross Priory	0.002	0.001			0.000	-

Muscle tissue was consistently enriched in ^{13}C relative to liver (Figure 3.16). This was the case at all sampling sites (Figure 3.17). However, muscle tissue was depleted in ^{13}C relative to bone. Bone stable isotope signatures were consistently enriched in $\delta^{13}\text{C}$ compared to liver and muscle tissues at all sites. Liver tissue was the most depleted in $\delta^{13}\text{C}$ and muscle tissue values lie between the two. Unlike $\delta^{15}\text{N}$, standard deviation values for $\delta^{13}\text{C}$ remained relatively constant within sites with the exception of Sallochy Bay.

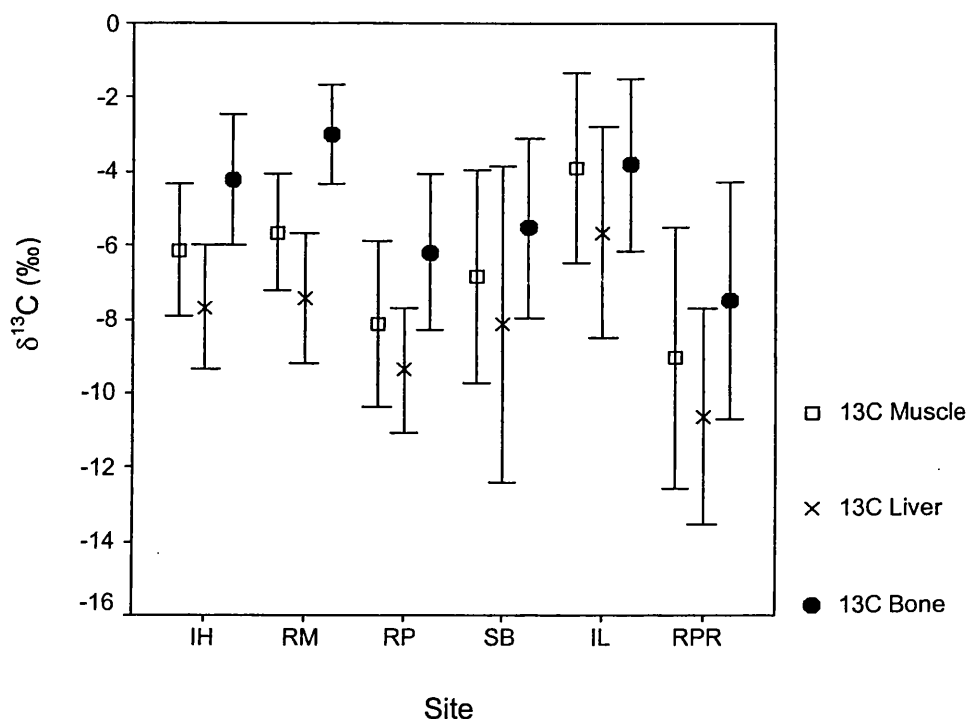


Figure 3.17 Mean $\delta^{13}\text{C} \pm 1$ standard deviation of muscle, liver and bone samples collected from ruffe at each of the sampling sites. Sites are ordered from north to south, Inverhoulin (IH), Rubha Mor (RM), Ross Point (RP), Salloch Bay (SB), Inchlonaig (IL) and Ross Priory (RPR).

3.6 Discussion

Within Loch Lomond there was significant spatial (between site) and seasonal variation in a range of physiochemical and community structure attributes. Amongst the physiochemical variables, there was a 5 fold variation in site exposure, a 12 fold variation in beach slope, 2.5 fold variation in BOD and an 8 fold variation in total phosphate concentrations (Table 3.1). Amongst the biological characteristics of the sites, there was variation in community species richness scores which ranged from 23-32, and also in community diversity scores which ranged from 1.8 – 2.7 between sites. There was also evidence of significant trophic variation in one ubiquitous fish species (the ruffe) between sites. Species richness scores of ruffe diet ranged from 2 and 12 and Shannon

richness scores of ruffe diet ranged from 2 and 12 and Shannon Weiner diversity scores also varied significantly between sites (range 0.02 to 0.59).

3.6.1 Are there differences in community structure between study sites?

The sampling sites within Loch Lomond were chosen as a result of differences in their physical properties. This diversity of habitats within Loch Lomond may create an opportunity for more spatial heterogeneity in community structure at particular sampling sites. It has been suggested that these differences in habitat complexity can have a strong influence upon community structure by affecting encounter rates between predators and prey (Jeffries and Lawton 1984; Werner and Gilliam 1984; Jeffries and Lawton 1985; Werner 1986). Within Loch Lomond, sampling sites in the north and south basins were similar in community structure however, the mid basin sites of Ross Point and Salloch Bay produced the most extreme results, lying at opposite ends of the diversity scale. It may be expected that diversity would be highest in the mid basin of the loch, as organisms here are not exposed to the more extreme physical conditions in the north and south basins. This would explain the high diversity values reported at Salloch Bay. Ross Point however, had the lowest of all diversity scores, despite its location in the mid basin of the loch. This site had an exposure value of almost double that of Salloch Bay which may explain the low diversity score.

Loch Lomond is a northern temperate loch and displays typical patterns of seasonality with different prey peaking in availability at different times (Keast 1979). Despite this, there was no clear pattern of change in community structure over the two sampling periods suggesting that the community structure at the

sampling sites examined within Loch Lomond remains relatively stable between seasons.

3.6.2 *Stomach Contents*

The availability of prey changes seasonally in temperate lakes (Keast 1979), and this was reflected in stomach contents of ruffe, with an increase in the number of species present between summer and winter and in some cases a doubling of this value was recorded between sampling periods. Ross Point had the lowest score and lowest variance possibly due to the relatively simple community structure at this site (Figure 3.11).

3.6.3 *Are there seasonal differences in isotopic signature between sites?*

The interpretation of stable isotope ratios relies on the assumption that the isotopic composition of the animal reflects that of its diet (Gannes et al. 1997). Trophic enrichment factors, from diet to tissue for carbon (1‰) and nitrogen (3.4‰) have been well established and applied in many investigations (DeNiro and Epstein 1978, 1981; Rau et al. 1983; Minagawa and Wada 1984; Vander Zanden and Rasmussen 2001). Within Loch Lomond, there were seasonal differences in the stable isotopic signature of ruffe between sampling sites. Over all sites the greatest seasonal variation observed in $\delta^{15}\text{N}$ was 3.19‰. Since the trophic enrichment factor for nitrogen has been established as 3.4‰ per trophic

level, the observed seasonal variation of 3.19‰ can be said to equate to 0.9 trophic level. In $\delta^{13}\text{C}$ the greatest variation observed was 4.79‰.

3.6.4 *Variation in isotopic signature between tissues*

The level of within tissue variation differed among the three tissue types analysed (muscle, liver and bone). The greatest variation was found for the $\delta^{13}\text{C}$ composition of liver. This is most likely due to the fast turnover of carbon in liver tissue where changes in isotopic composition of the diet can be reflected in the liver within days (Gaston and Suthers 2004). Bone had the least variation of all tissues for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, probably due to the relatively slow turnover rate of bone (2-10 years, (Burnstead 1985)).

Chapter 4. Variation in $\delta^{15}\text{N}$ as a Measure of Trophic Niche Width

The aim of this chapter was to explore a novel technique for estimating trophic niche width using the population standard deviation of measurements of the stable isotope ratios of N ($\delta^{15}\text{N}$). The possible strengths and some weaknesses of this approach are discussed and the use of standard deviation of $\delta^{15}\text{N}$ is tested against more traditional estimates of trophic niche width such as stomach contents analysis. The following hypothesis are explored:

- There are significant potential strengths in using $\delta^{15}\text{N}$ as a measure of trophic niche width. These are:
 - $\delta^{15}\text{N}$ integrates over a more ecologically realistic time scale compared with more traditional methods such as diet estimated from stomach contents analysis which provide only an instantaneous 'snapshot' of dietary preferences.
 - It provides a single scale over which differing diets can be compared. This allows direct comparison amongst individuals, populations, and species through the arrangement of species along a single diversity scale.
 - Data collection can be fast and potentially non-destructive in some species.
 - The integration period can be modified by choosing different tissue types. Liver integrates over a number of days (Hobson and Clark

1992a; Gaston and Suthers 2004), muscle integrates over weeks (Hobson and Clark 1992a; Gaston and Suthers 2004) and bone integrates over several months or years (Bumstead 1985; Hobson and Clark 1992b).

- Using a simple but realistic model of likely standard deviations in $\delta^{15}\text{N}$ (gathered from the literature), it is possible to produce estimates of the statistical precision of limits of change using $\delta^{15}\text{N}$ as an index of trophic niche width. Based on this model, it is predicted that:
 - Foraging specialist populations (with low standard deviation in $\delta^{15}\text{N}$) would require a smaller numerical change in n standard deviation than would a generalist population in order to be detectable
 - The top 20% of each of the tails of the normal distribution of diet variability would have to shift by at least 0.068 trophic levels in extreme specialist and 0.232 trophic levels in an extreme generalist population to be statistically detectable.

The ability of $\delta^{15}\text{N}$ to predict more traditional methods of trophic niche width measurement is explored by comparing standard deviation in $\delta^{15}\text{N}$ of ruffe tissue from the six Loch Lomond sites with a more traditional measure of trophic niche width (the Shannon-Weiner Index of stomach contents).

Having established a sound basis for the use of $\delta^{15}\text{N}$ as a measure of niche width the following was then carried out.

- An examination of the possibility that variance in $\delta^{15}\text{N}$ can be used to separate population from individual generalism.
- Empirical data was used to test the relationship between trophic niche width and variance in $\delta^{15}\text{N}$.
- The potential precision of the use of $\delta^{15}\text{N}$ for the estimation for trophic niche width was quantified using published data.

4.1 Introduction

The theoretical basis for the use of $\delta^{15}\text{N}$ as a means of estimating trophic niche width in consumers is explored. A part of this chapter forms the basis of a published paper (see Appendix 1).

4.1.1 *Trophic niche width and its estimation*

The term niche was used by (Hutchinson 1957) to describe the range of physical and biological conditions, including limiting resources, required by a species in order to maintain a stable or increasing population size. Hutchinson's definition is that of an n-dimensional hypervolume, where the n dimensions correspond to independent physical and biological variables that affect the abundance of that species. The range of values of each of these variables within which the individual or population can persist is known as niche width.

Trophic niche explored through conventional dietary analysis is the most reliable and frequently studied element of niche space (Hyslop 1980; Bearhop et al. 2004). Dietary analysis provides detailed information on the range of prey items consumed by individuals and has the potential to yield information on prey

preferences of the study organism (Post 2002). However, this type of dietary analysis does have a number of drawbacks (Zerba and Collins 1992; Schindler et al. 1997). A major disadvantage is that it provides only an instantaneous snapshot of dietary preference at a particular point in time (Grey 2001), yet many animals are highly opportunistic foragers and their diet may vary substantially over time (Pinnegar and Polunin 1999; Post 2002). Furthermore, the method is cumbersome and labour-intensive, and under-estimates and over-estimates of the relative abundance of prey items are common since the technique does not account for differing rates of digestibility or differences in prey assimilation rates (Grey 2001; Bearhop et al. 2004). An alternative to dietary analysis, which is used increasingly by ecologists to determine trophic relationships, is that of stable isotope analysis (Peterson and Fry 1987; Hobson and Wassenaar 1999). Although this technique does not give the detailed picture of dietary preferences provided by gut content analysis, stable isotope analysis can provide an average estimate of an organisms preferred diet which is much less subject to temporal bias and also takes into account not just ingested but assimilated food (Hesslein et al. 1993; Post et al. 2000).

The problems associated with determining trophic relationships in natural ecosystems are a major obstacle to our understanding of ecosystem processes (Paine 1988; Yoshioka and Wada 1994). In order to produce a useful and robust alternative measure of trophic niche width, there are a number of criteria which should be met. Firstly, it is essential that a direct comparison along a single diversity scale of individuals, populations and species can be made regardless of their location, dietary preference or trophic level (Bearhop et al. 2004). For example, it is usual to find studies of stomach content analysis to include dietary items identified to class, family, genus and species mixed within a single study (Vander Zanden et al. 1999), thus real dietary diversity is difficult to determine.

This is the criterion least frequently met by current techniques and is a stumbling block to comparative studies. Secondly, the ability to determine the diversity of dietary composition with regards to richness and evenness of assimilated dietary components is also very important. Finally, as an organism's diet may display both spatial (France 1995; Vander Zanden and Rasmussen 1996) and temporal (Post 2002) variation with changing season and stage of development (Magnan and FitzGerald 1984; Werner and Gilliam 1984; Werner 1986), the integration of dietary information over different time scales, preferably from a single sampling event is important (Bearhop et al. 2004).

4.2 The use of stable isotope analysis to determine trophic niche width

Stable isotope ratios of nitrogen and carbon have been used increasingly by ecologists to elucidate patterns in food webs (Peterson and Fry 1987; Kling et al. 1992; Cabana and Rasmussen 1994; Bearhop et al. 2004; Grey et al. 2004). Despite this, little attention has been given to the variation associated with mean isotopic signatures within communities (Bearhop et al. 1999; Genner et al. 1999). The potential application of this variance in stable isotope signature as a measure of trophic niche width is theoretically sound and simple to use (Bearhop et al. 2004). The technique meets all the criteria set out in section 4.2 and has the potential to produce a dynamic measure of trophic niche breadth.

Within any community, one would expect to see a degree of variance in individual stable isotope signatures around a group mean (Bearhop et al. 1999). The extent

of the variance exhibited by a community will be governed by the following factors.

1. The range of isotopically distinct prey species consumed. In general, a population of animals which consume a wide range of prey species would be expected to exhibit a wider variation in isotopic signatures than those consuming a narrow range of prey.
2. The evenness of prey components in the diet over time is also important. Populations where individuals consume widely differing proportions of each of their prey items over time would be expected to show more variation in tissue stable isotope ratios than those which consume a consistent proportion of each prey.
3. The range of trophic levels from which prey is drawn also affects variance. Populations where individuals consume prey from a broad spectrum of trophic levels would be expected to show more isotopic variance than those which feed on prey species drawn from one trophic level
4. Geographic foraging area would also be expected to play a role in determining variance in stable isotope signature. Studies have demonstrated that spatial differences in stable isotope signatures are commonplace, even within single lake ecosystems (Angradi 1994; France 1997; Vander Zanden et al. 1999). Since this variation at the base of the food web will propagate through to higher levels, populations where individuals forage in a range of geographic areas would be expected to show more variation in the stable isotope signatures of their tissues than those from less mobile populations.

5. Stable isotope signatures can also be affected by variability in individual physiology. Physiological differences among individuals within a population, or within the same individual over time will result in variance in tissue isotope signature. For example variability in metabolic rates may lead to inter-individual variation in tissue isotope signatures (observed during studies of captive individuals, (Hobson and Clark 1992b; Bearhop et al. 2002). Furthermore, it has been reported that the tissues of individuals in poor nutritional condition have elevated ^{15}N compared to those of individuals of better condition (Hobson et al. 1993). It is likely that the influence this may have on population or serially sampled individual variance will be small and will manifest in noise, rather than causing error between populations or individuals. Nevertheless, more work is required in this area as despite recent advances, our understanding of how variability in physiology influences tissue stable isotope signatures is still limited.
6. Finally, variability in diet-tissue fractionation must also be considered. Diet-tissue isotopic fractionation may vary with the type of food being consumed (France and Peters 1997; Vander Zanden and Rasmussen 2001; Post 2002; McCutchan et al. 2003) or through differential mobilisation of stored resources (Adams and Sterner 2000). However, since enrichment factors for the same tissue type fuelled by different diets differ by up to 2‰ for $\delta^{15}\text{N}$ and by just over 1‰ for $\delta^{13}\text{C}$ (Hobson and Clark 1992b; Haramis et al. 2001; Bearhop et al. 2002), this degree of variability ought only to account for a large proportion of the variance when the dietary isotopic variance is small.

In order that variance in mean isotopic signature can be used as a useful measure of trophic niche breadth, there are a number of conditions which must be met.

1. It is essential that the isotopic signature of prey items available to consumers exhibit differences in stable isotope signature. If this variation were not evident, further consideration of niche width (through examination of consumer isotopic variance) would be useless (Matthews and Mazumder 2004). The validity of this assumption can be verified by direct measurement of the isotopic signature of potential prey items (Matthews and Mazumder 2004).
2. Isotopic signatures and diets of prey species should remain relatively constant over time at the base of the food web. Studies (Zohary et al. 1994; Post 2002; Matthews and Mazumder 2003) have demonstrated that baseline isotopic signatures can be affected by temporal shifts in nutrient inputs and primary production. Furthermore, dietary preferences of the target species for particular prey items may change over time (Post 2003). As long as the variance caused by these elements is less than the variance resulting from a consumer dietary shift (revealed by sampling of prey items), stable isotope variance is likely to remain a robust measure of trophic niche width.

Adopting the appropriate tissue (i.e. bone, liver or muscle) for stable isotope analysis is important because tissues and tissue components differ in turnover rate, and hence, the temporal resolution for dietary analysis differs among them (Tieszen et al. 1983; Schoninger and DeNiro 1984; Sholto-Douglas et al. 1991; Hobson and Clark 1992b; Hesslein et al. 1993; O'Reilly and Hecky 2002). In a

population of generalists, variability in diet amongst individuals would be expected to exist at only shorter temporal scales, and this variation is likely to become lost through averaging of stable isotope signature over longer periods. In such a situation, tissues with integration times shorter than the period of niche width assessment will likely provide a better indicator of niche width. For example, where a whole population shifts diet synchronously is to be compared with another population that doesn't, serial sampling of tissues which integrate relatively short-term information would be required. As is the case when employing more traditional approaches to the study of trophic niche width (Bearhop et al. 2004), the detail of the question being asked will determine the most appropriate choice of tissue.

4.3 Is it possible to discriminate between population and individual generalism?

Where a generalist feeding strategy is identified, it is important to attempt to determine whether the pattern is evident for the population as a whole or purely on an individual basis. Two types of generalist feeding have been identified. A Type A generalist population is characterised by generalist individuals all taking a wide range of food types, whereas a Type B generalist population is composed of individuals each specialising on a different but narrow range of food types (Van Valen 1965; Grant et al. 1976). Using conventional methods to address this problem has required labour intensive field observations and often populations of identifiable individuals (Bearhop et al. 2004). However, either by serial sampling or utilising the differential rate of tissue turnover, stable isotope analysis potentially offers a powerful approach to consider the extent of population, or

individual generalism with relative ease. Since different animal tissues integrate dietary signatures over different temporal scales (Hobson and Clark 1992b; O'Reilly and Hecky 2002), in a population of generalists, variance among tissues that integrate diet over short temporal scales (shorter than the period of trophic variation), should be larger than the variance for tissues that integrate diet over longer temporal scales (that cover the period of trophic variation). Thus for example, tissues that integrate over days and weeks such as blood plasma, blood cells or liver tissue (Hobson and Clark 1992; Hesslein et al. 1993; Gaston and Suthers 2004), are much more likely to discriminate dietary generalism than are tissues which integrate variation over the life-time of the animal such as bone, groups of feathers, fish otoliths or scales (Schoninger and DeNiro 1984; Sholto-Douglas et al. 1991). It follows that, for a population of specialists, one would predict little or no change in variance between long- and short-term integrators.

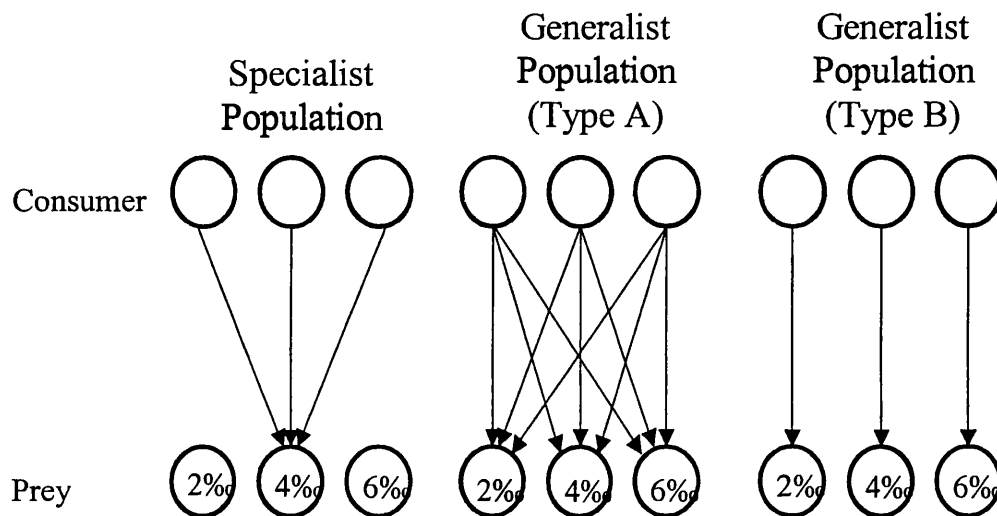


Figure 4.1 Illustration of the mechanisms by which three different populations may achieve the same isotopic signature of 8‰ made up of the mean signature of the prey population (4‰ in all cases) plus the enrichment factor of 4‰

If we assume that diet/tissue fractionation is constant at 4‰, that prey isotope ratios remain constant over time and that Type A individuals consume all prey types in equal amounts then there are potentially three sampling regimes that could enable the use of stable isotope variance in animal tissue to discriminate between Type A and Type B generalism when food is consumed as illustrated in Figure 4.1.

- Sampling a tissue that integrated dietary information over long temporal scales would allow identification of a population of Type B generalists and would likely give consumer population values (mean \pm s^2) similar to those in Table 4.1.

Table 4.1 Isotopic signature and variance of populations sampled using tissues which integrate over long temporal scales used to identify Type B generalists.

Specialist	Generalist Type A	Generalise Type B
8‰ \pm 0	8‰ \pm 0	8‰ \pm 4

- Sampling a tissue that integrated dietary information over short temporal scales (with a large sample size) would allow identification of a population of specialists and would likely give consumer population values (mean \pm s^2) similar to those in table 4.2.

Table 4.2 Isotopic signature and variance of populations sampled using tissues which integrate over short temporal scales used to identify specialists.

Specialist	Generalist Type A	Generalise Type B
$8\text{‰} \pm 0$	$8\text{‰} \pm 4$	$8\text{‰} \pm 4$

- Assuming that the tissue being sampled integrates dietary information over a shorter period than that which diet varies over, serial sampling the same tissue (integrating very short-term dietary information, such as blood plasma samples, or short sections from feathers, hair or possibly whiskers) from the same individual over time would allow identification of a Type A generalist population and would likely give individual values (mean \pm s²) similar to those in table 4.3.

Table 4.3 Isotopic signature and variance of populations sampled using tissues which integrate over a shorter period than that over which diet varies used to identify Type A generalists.

Specialist	Generalist Type A	Generalise Type B
$8\text{‰} \pm 0$	$8\text{‰} \pm 4$	6, 8 or $10\text{‰} \pm 0$

4.3.1 *An empirical test of variance in $\delta^{15}\text{N}$ as a measure of trophic niche width*

Sampling sites selected from within Loch Lomond (see chapter 3) were shown to conform to the general requirements set out in section 4.3 for $\delta^{15}\text{N}$.

As a result, the variance (expressed as the standard deviation) in mean $\delta^{15}\text{N}$ isotopic signatures of ruffe populations from each of these sampling sites was used to test the hypothesis that variance in stable isotope signature is a robust measure of trophic niche breadth. The Shannon-Weiner index of stomach contents (a measure of trophic niche width) was calculated for ruffe populations at each of the sampling sites. These stomach contents Shannon-Weiner values were then regressed on the variance in mean $\delta^{15}\text{N}$ values of ruffe muscle tissue (Figure 4.2). Shannon-Weiner scores and variance in $\delta^{15}\text{N}$ were calculated for ruffe populations at each of the sampling sites in both summer and winter.

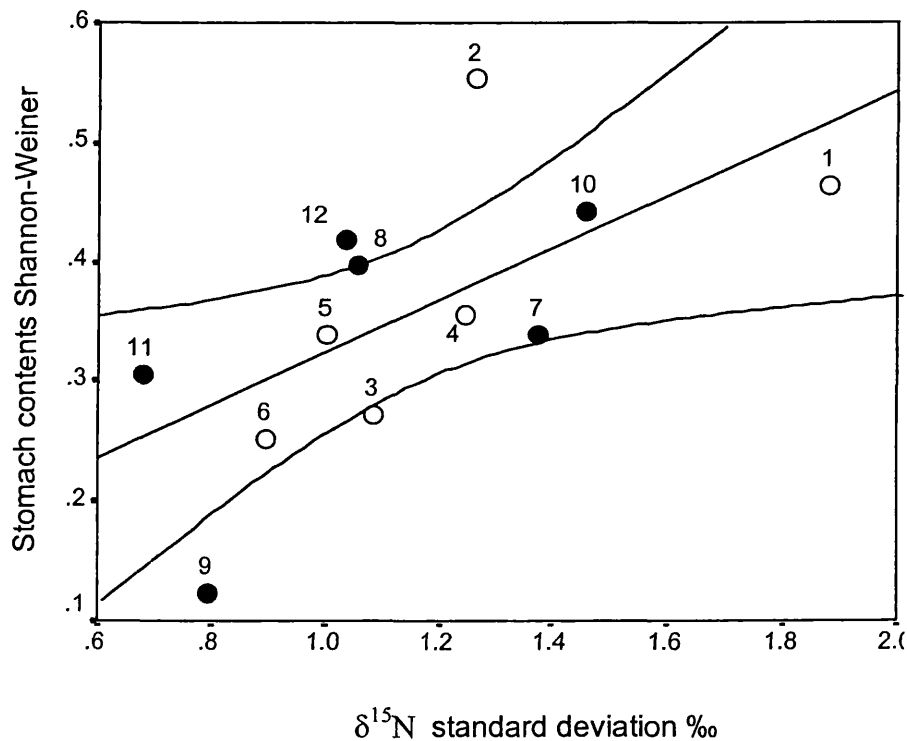


Figure 4.2. Standard Deviation in Ruffe Muscle $\delta^{15}\text{N}$ and Ruffe Stomach Contents Shannon-Weiner Based on Site Mean Values Showing 95% CI. Open circles indicate winter sampling sites and filled circles indicate summer sampling sites. Sites are coded as follows. 1 and 7 = Inverhoulín, 2 and 7 = Rubha Mor, 3 and 9 = Ross Point, 4 and 10 = Sallochay Bay, 5 and 11 = Inchlonaig and 6 and 12 = Ross Priory.

Regression of $\delta^{15}\text{N}$ against the Shannon-Weiner of ruffe stomach contents was significant ($F_{1,10} = 6.654$, $P = 0.027$) and explained 40% of the variation in stomach contents Shannon-Weiner scores. The regression generated the regression model:

$$\text{Stomach contents Shannon-Weiner} = (0.220 * \delta^{15}\text{N}) + 0.104$$

This demonstrates that the standard deviation of $\delta^{15}\text{N}$ of ruffe muscle tissue predicted the diversity of stomach contents, and supports the hypothesis that the standard deviation of $\delta^{15}\text{N}$ is a realistic measure of trophic niche width in ruffe.

4.4 The precision of detectable change in variance

– a modelling approach

For variance in stable isotope signatures to be a useful tool for quantifying niche width, we must be able to detect changes in tissue stable isotope signatures over time, or differences between sites or populations. To determine the sensitivity of isotopic variance as a measure of niche width, one is required to model the detection limits for a range of realistic probable measures of variance (standard deviation) in the stable isotope signatures of animal tissues.

Clearly the ability to detect a significant change in standard deviation over time, or difference between populations, is dependent on the magnitude of the change/difference with respect to the size of the two standard deviations. The F-distribution provides the theoretical probability that two measures of sample variance are drawn from a normal population distribution with the same variance. This variance ratio test (F-test) provides a method by which it is possible to calculate the minimum change in a standard deviation of, in this example, $\delta^{15}\text{N}$

that would be statistically significant. In this instance the example of feather analysis of stenophagus shags *Phalacrocorax aristotelis* (L.) with a relatively narrow trophic niche width ($\delta^{15}\text{N}$ standard deviation 0.57 ‰) and polyphagus cormorants *Phalacrocorax carbo* (L.) with a relatively broad niche width ($\delta^{15}\text{N}$ standard deviation 2.01 ‰) (Bearhop et al. 1999), was examined. A probability level of $p=0.05$ and a realistic but moderate sample size of 20 was applied to the data to determine the minimum change in $\delta^{15}\text{N}$ standard deviation required to be statistically detectable in the analysis of trophic niche width using stable isotope analysis.

4.5 Model output

For a population where the standard deviation is initially low (e.g. the shag), the difference between standard deviations would need to be at least 0.27 ‰ to be significant (for $p=0.05$, $N=20$). Trophic enrichment factors have been established for nitrogen where an increase of one trophic level is equivalent to an increase in the nitrogen signature of a consumer of 3.4‰ (Minagawa and Wada 1984; Vander Zanden and Rasmussen 2001). Based upon this, a difference between standard deviations of 0.27 equates to a dietary shift of 0.079 of one trophic level. For a population where the standard deviation of the population is initially high (e.g. the cormorant), the difference between standard deviations would need to be 0.94 ‰, which equates to a dietary shift of 0.276 of one trophic level, (for $p=0.05$, $N=20$) to be statistically detectable. Thus the technique is potentially more sensitive than the limits of the analytical instrumentation (precision is typically 0.2 to 0.3 ‰ for $\delta^{15}\text{N}$). The theoretically required change in the shape of the normal distribution for both of these populations are shown in Figure. 4.3.

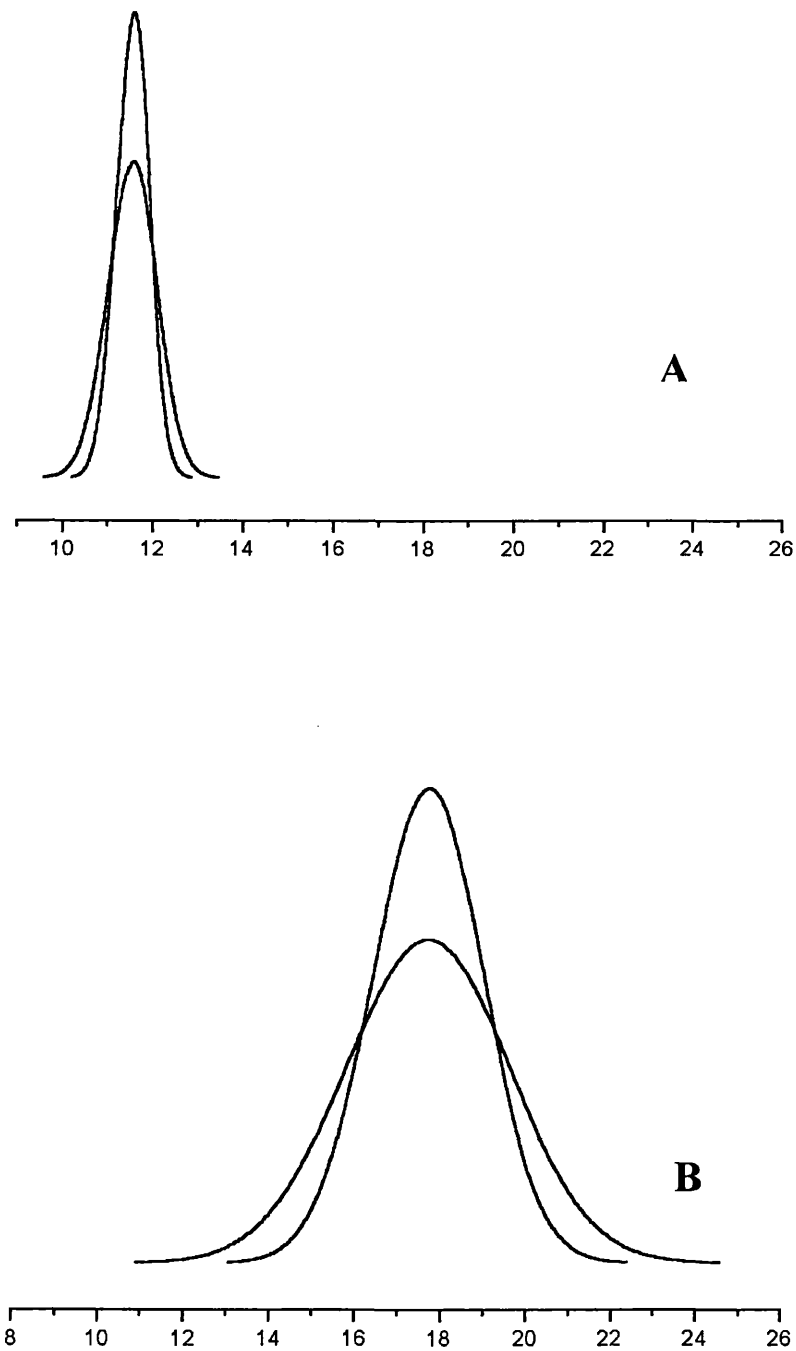


Figure 4.3 The chance in standard deviation of the normal distribution that is statistically detectable ($P=0.05$) for a sample size of 20, for (A) a specialist population with a small initial standard deviation (shags S.D. = 0.57) and (B) for a generalist population, with an initially high standard deviation (cormorants; S.D.= 2.01).

Assuming that the tissue $\delta^{15}\text{N}$ signatures in the population remains normally distributed, the z-distribution can be used to predict the change in $\delta^{15}\text{N}$ that would be required for individuals at different positions within the normal distribution to generate the required change in standard deviation. Thus, for the lowest possible detectable change in standard deviation from the shag population for a sample size of 20 and a 5% probability, the 1% of the population that deviates most from the mean (i.e. at the extreme tails of the normal distribution) would be required to become higher in the upper tail and lower in the lower tail by 0.62 ‰ or 0.182 of one trophic level. Similarly 20% of the population in each tail would need to deviate by 0.23 ‰ which equates to 0.068 of one trophic level (Figure. 4.4) and so on. In contrast, for the cormorant population with an initially broad standard deviation, for $N=20$ and $P=0.05$, 1% of the population in each normal distribution tail would be expected to deviate by 2.16‰ which is 0.636 of one trophic level and 20% by 0.79 ‰ or 0.232 of one trophic level. Stable isotope variance as a measure of niche width therefore appears sensitive to relatively subtle changes in population niche width, where only a proportionally small number of individuals alter their behaviour, a response that may be predicted for resident species in a system where alien introductions have occurred (Kidd et al. 1998). Such effects have proved difficult to detect conventionally, although the use of stable isotope tissue signatures as indicators of trophic status and foraging area have provided insights into the impacts of introduced predatory fish upon North American lake biota (Vander Zanden et al. 1999).

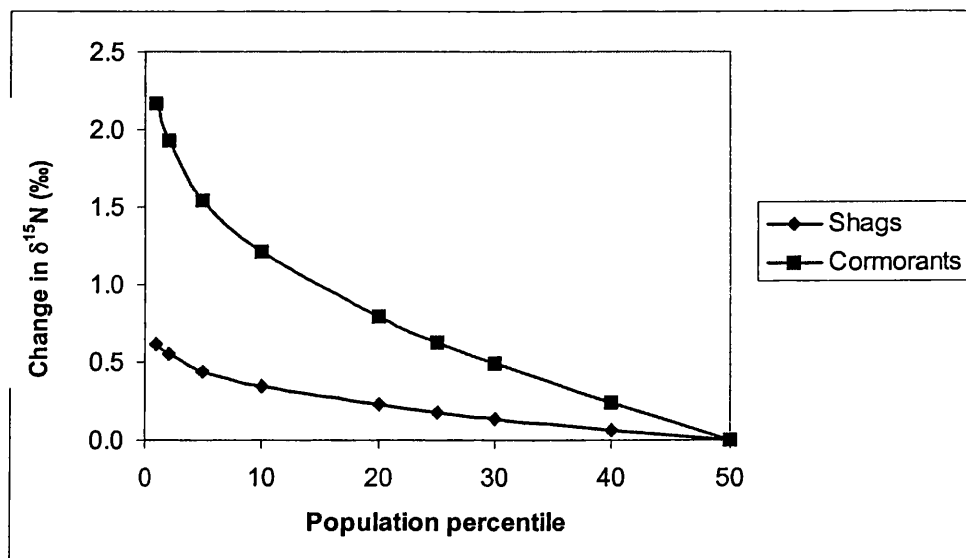


Figure 4.4 Statistically detectable change ($P=0.05$; $N=20$) in $\delta^{15}\text{N}$ for individuals at different starting points in the normal distribution (expressed as population percentage deviations from the mean) for a generalist feeding population (cormorants) and a specialist feeding population (shags).

The absolute change in $\delta^{15}\text{N}$ required in a normal distribution at the extremes of the distribution depends upon the initial standard deviation of the population. Figure 4.5 shows the relationship between position in the normal distribution and change in $\delta^{15}\text{N}$ required for a range of starting standard deviations for a sample size of 20 and a probability of 0.05. Thus, for a realistic range of initial population $\delta^{15}\text{N}$ and standard deviations from 0.5 ‰ to 2.5 ‰ (indicating narrow vs. relatively wide trophic niche width), the change in $\delta^{15}\text{N}$ required by the 10% on each tail of the population normal distribution in terms of trophic level, ranges from 0.087 to 0.440 of one trophic level. Clearly the absolute sensitivity of the technique to determine niche width change is dependent upon initial niche width. However the magnitude of dietary change required in individuals even at large but realistic starting standard deviations is feasible: The shift in $\delta^{15}\text{N}$ of assimilated diet

required in 1% of the extremes of the population approximates 0.8 of one trophic level, which is equivalent to a shift in isotopic signature of 2.7‰.

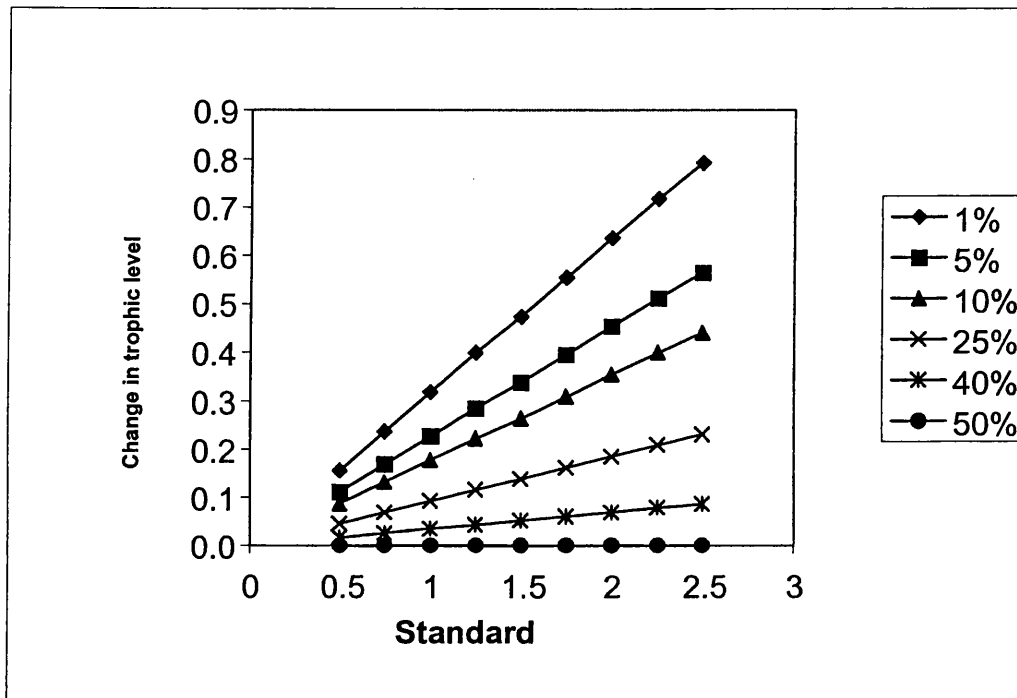


Figure 4.5 Statistically detectable ($P=0.05$; $N=20$) changes in trophic level for individuals in a normal distribution expressed as the population percentage deviation from the mean for each of the tails in a normal distribution for starting standard deviations ranging from 0.5 to 2.5.

4.6 Discussion

Here it has been shown that there is a good theoretical basis for the use of the standard deviation of $\delta^{15}\text{N}$ as an indicator of trophic niche width and that this technique may have significant advantages over more traditional techniques which:

- Rarely lie along a single scale making comparisons between populations or species difficult;
- Have difficulty in combining dietary prey diversity and evenness in an ecologically meaningful way;
- Fail to integrate diet over ecological timescales thus usually only comprise single snapshots of niche width.
- Tend to be rather cumbersome and labour intensive

Also empirical data from Loch Lomond (Figure 4.2) supports the hypothesis that $\delta^{15}\text{N}$ standard deviation is as good a predictor of niche width as that measured using more traditional techniques. Here it has been demonstrated that the standard deviation of a population $\delta^{15}\text{N}$ correlates with the diversity of the stomach contents of the same population.

There is also good reason to believe that this technique can discern between two very different types of generalism (Type A and Type B), either by serial sampling or by utilising the differential rate of tissue turnover (Figure 4.1). A Type A generalist population is characterised by generalist individuals all taking a wide range of food types, whereas a Type B generalist population is composed of individuals each specialising on a different but narrow range of food types (Van Valen 1965; Grant et al. 1976).

Where individuals within the population were identifiable, serial sampling from the same individuals could distinguish Type A and Type B generalists. Serial sampling could comprise multiple blood samples, sampling sections of feathers

grown at different times in the moult cycle or sampling multiple sub-sections of long hairs such as vibrissae. In the case of fish, where non-destructive sampling is not possible, tissues of differing turnover rates such as liver, muscle and bone will provide similar information. When such a sampling regime is employed the variation exhibited by individuals from a Type A generalist population will be approximately equal to that exhibited by the population as a whole. On the other hand, the variation exhibited by an individual displaying Type B generalism will be low relative to the variation displayed by the population as a whole (Figure 4.1 and Table 4.3).

The difference in Type A and Type B generalists can be discerned by utilising the differential rate of tissue turnover as follows. In a population of generalists the variance among tissues that integrate diet over short temporal scales (shorter than period of trophic variation), will be larger than the variance for tissues that integrate diet over longer temporal scales (that cover the period of trophic variation). However, tissues which integrate over days and weeks, (such as blood plasma, blood cells or individual feathers (Hobson and Clark 1992b; Hilderbrand et al. 1996; Bearhop et al. 2002), are much more likely to discriminate dietary generalism than tissues which integrate variation over much longer time scales such as bone, groups of feathers, fish otoliths or scales (Hobson and Clark 1992a; Begg and Weidman 2001). In this instance, a population of specialists would display little or no change in variance between long- and short-term integrators (Figure 4.1, Table 4.1 and Table 4.2).

For variance in stable isotope signatures to be a useful tool for quantifying niche width, it must be possible to detect changes in tissue stable isotope signatures over time, or differences between sites or populations. The ability to detect change in $\delta^{15}\text{N}$ standard deviation depends upon the initial population standard

deviation in $\delta^{15}\text{N}$ (i.e. the initial niche width). Here it has been demonstrated that changes in populations with a narrow niche width are easier to detect than changes in populations with a wide niche width starting point. Despite this, it has been demonstrated that the shift in $\delta^{15}\text{N}$ of assimilated diet required in 10% of the extremes of a population with a $\delta^{15}\text{N}$ standard deviation of 2.5 approximates 0.8 of one trophic level, which is equivalent to a shift in isotopic signature of 2.7‰ demonstrating that the magnitude of dietary change required in individuals even at large but realistic starting standard deviations is feasible. This demonstrates that the use of variation in population $\delta^{15}\text{N}$ as a measure of trophic niche width is a robust technique that is relatively easy to employ and offers many advantages over traditional methods.

Chapter 5. Causes and Consequences of Variation in Trophic Niche Width

In this chapter a number of hypotheses are explored related to the effect of community structure on trophic niche width in ruffe. In addition, an examination is made of the possible consequences of variation in trophic niche width for individuals at different study sites.

- In this study there was a significant relationship between competition and community complexity
- However, there was no evidence of the hypothesised relationship between community competition and trophic niche width
- There was also no evidence for a relationship between maximum trophic chain length and trophic niche width
- Nor was there evidence for an effect of community complexity on trophic niche width
- Physiochemical characteristics such as BOD and phosphate levels of the sampling site were shown to affect trophic niche width
- There was no significant relationship between community characteristics of sampling sites and trophic niche width
- There was no evidence for the hypothesised relationship between niche width and individual fitness

5.1 Introduction

Hutchinson (1957) used 'niche' to describe the range of physical and biological conditions required by a species in order to maintain a stable or increasing population size. Hutchinson's definition is that of an n-dimensional hypervolume, where the n dimensions controlling niche width correspond to independent physical or biological variables that affect the abundance of that species.

The constraints imposed upon the niche width of a particular community by such factors are of two distinct types.

- Abiotic constraints originating in the external environment and,
- Biotic constraints arising from interactions with other species in the same environment.

Within the abiotic category of constraints, the niche width of an organism is regulated by physical needs alone. If these needs are met then the organism is able to maintain a stable population. This is described as the fundamental or pre-interactive niche of a species (Hutchinson 1957). The observation that a species does not always occur in an area where conditions are within acceptable limits and where necessary resources are available suggests that there are other factors controlling its presences or absence. The occurrence of a particular species in an area may be precluded by the action of individuals of other species which compete with it or prey upon it. This is referred to as the realised or post-interactive niche (Hutchinson 1957) and describes the situation where a species is forced to share resources with any number of interacting organisms in the community. Thus realised niche for any species is likely to be a function of both

the physical environment and the biotic characteristics at any site (Hutchinson 1957).

Therefore, where changes in niche width and/or trophic position are observed as a function of changing community structure, it is predicted that there would be implications for the fitness of individuals within that community.

Here the potential biotic and abiotic factors leading to modification of trophic niche width, expressed as variation in $\delta^{15}\text{N}$ in a community, are explored and the implications of niche modification on community fitness are assessed. Specifically the following questions are tested:

- Does competition increase with increasing community complexity?

Although largely untested, the scope for competitive interaction is likely to be significantly affected by the community to which any organism is exposed and more specifically the structure of the food web. For example, food web complexity may affect the trophic position and foraging niche width of a species (Bolnick 2001). Food webs with a high level of complexity contain a large number of interacting species, providing greater scope for modification of the trophic position of a single species and for a reduced foraging niche width (Bolnick 2001). Theory would also predict that this effect would not occur in all species equally, with the effect of community complexity upon competition increasing with the degree to which species are capable of foraging upon a wide range of food (Connell 1961; Byers 2000).

- Is trophic niche width affected by community competition?

Competitive exclusion and niche separation theory predicts that for any organism, the foraging niche determined by the basic functional feeding morphology constraints of the species (the “fundamental foraging niche”) will be modified by competitive interaction with other species (Connell 1961). Thus the actual trophic position that any species takes within a community (the “realised feeding niche”) is determined by competition operating upon functional morphology constraints (Hutchinson 1957). Competition will lead to diversification by depressing fitness of individuals using the original resource to the point where previous sub optimal resources have a higher value (Bolnick 2001). High levels of intraspecific competition will lead to niche expansion while interspecific competition will cause niche width to contract (Bolnick 2001).

- Does community complexity affect trophic niche width in a single predator species?

Theory predicts that community complexity will have an affect upon trophic niche width since the diversity of feeding categories available to a predator will increase with increasing species richness. A greater range of food resources will be available in more species rich communities, leading to an increase in feeding niche width (Hilldrew 1992).

- Do the physical characteristics of a foraging site affect the trophic niche width expressed within a species?

In lakes, a greater diversity of habitat will create an opportunity for a more spatially heterogeneous community (Vander Zanden et al. 1999). Structurally complex environments are often associated with higher

abundances and diversity of invertebrate resources which are important for benthivorous fish such as ruffe (Gilinsky 1984).

- Do biological characteristics of a foraging site affect the trophic niche width expressed within a species?

Since the publication of Hairston *et al.* (1960), a major topic of interest in ecology has been that of species interactions and how these interactions can be used to predict community dynamics (Paine 1980; Pimm 1982; Carpenter *et al.* 1985; Carpenter *et al.* 1987; Persson *et al.* 1988). If the physical needs of an organism are met, the organism is able to persist in that environment. However, biological aspects of the foraging site such as the occurrence of additional species which occupy the same general region of the n -dimensional hypervolume, will lead to reductions in the size of the organisms realised niche (Hutchinson 1957).

- Are biological or physical characteristics more important in explaining niche width?

Both physical and biological factors contribute to the modification of trophic niche width, however it may be that one of these factors exerts a larger influence on niche width.

- Does invertebrate density affect trophic niche width?

A detailed study of the guild of detritivorous stoneflies (Plecoptera) in four streams differing in species richness has provided evidence that density compensation occurs, niche width decreases and niche overlap declines as species richness increases (Hilldrew 1992).

- Does expressed trophic niche width affect fitness of individuals within a community?

Here it is hypothesised that the body condition of fish in a population will be affected by variations in community complexity, competition, and niche width, leading to changes in individual survival and or fecundity (Begon et al. 1996). Furthermore, it is hypothesised that changes in the level of community complexity, competition and trophic niche width typically lead to changes in rates of resource intake per individual, and thus to decreased rates of individual growth or development, or perhaps to decreases in amounts of stored reserves.

The annual cycle for temperate fish species in a lake such as Loch Lomond can be divided into warm 'growing' and cold 'non-growing' seasons (Conover 1992). In the summer, environmental conditions permit body size and energy stores to increase (Griffiths and Kirkwood 1995). These energy stores are built up over the summer and may be consumed in maintenance over the winter (Newsome and Leduc 1975; Pierce et al. 1980). Overwinter mortality in some species has been linked to exhaustion of energy stores (Newsome and Leduc 1975; Post and Evans 1989; Adams and DeAngelis 1987). It is known that for some fish species lipid stores are critical to overwinter survival and individuals will radically alter foraging to maintain sufficient lipid levels for survival (Metcalf and Thorpe 1992). In this programme of work, ruffe body size (measured as fork length and weight) and lipid deposition levels were used as proxies for fitness.

5.2 Methods

5.2.1 *Selection of Study Sites*

Generally, niche width studies involve the comparison of systems that are ecologically similar, contain similar communities and are located within a restricted geographic region (Smith and Smith 2001). Although this goes some way to address the problems of making comparisons between systems, there are still some inherent differences that may confound results. Loch Lomond is an ideal site to study niche width since, as a result of its division by the Highland Boundary Fault, this loch has in a single water body, 3 basins of very different nutrient status, the impact of which can be examined without the confounding effects of comparisons between different water bodies. In addition, pilot studies have shown that the extensive habitat heterogeneity found in the littoral zone of Loch Lomond causes significant modification of invertebrate community structure and population size (Adams, unpublished data), and this information can be utilised to test hypotheses relating to community structure.

5.2.2 *Selection of a Study Species*

In order to investigate trophic niche width and the factors controlling it, it was essential to identify a species with the potential to modify its niche as a result of the environment to which it was exposed. It was also essential to study a species that was ubiquitous throughout Loch Lomond and available in both summer and winter. The ruffe *Gymnocephalus cernuus* fulfils all these criteria. Ruffe were first discovered in Loch Lomond in 1982 (Adams and Maitland 1998) and are thought

to have been introduced into the loch by anglers who used them as live bait for fishing of northern pike *Esox lucius* (Maitland and Lyle 1991). Ruffe in Loch Lomond are benthivorous omnivores, and are extremely catholic in their diet, which includes a wide range of invertebrates, fish ova and fish (Johnson 1965; Adams and Tippet 1991). There is also evidence that the diet of ruffe changes both geographically and seasonally within Loch Lomond (Adams and Maitland 1991). These fish are also very hardy and able to function in a wide range of environments. They have a relatively high temperature tolerance range with an upper lethal limit of 31°C (Varley 1967) yet they can maintain foraging in water temperatures at least as low as at least 4°C (Bergman 1987; Adams and Tippet 1991). They also have a short generation time (Muss and Dahlstrom 1967; Varley 1967) and high fecundity (Muss and Dahlstrom 1967; Varley 1967). The age at which 50% of individuals spawn in a stable ruffe population has been reported as 1 year old for males and 2 years for females (Muss and Dahlstrom 1967; Varley 1967) which allows them to respond rapidly to the environment in which they live. The traits listed above make ruffe an ideal study species to investigate trophic niche width in Loch Lomond.

5.2.3 *Collection of samples for stable isotope analysis*

Invertebrate and fish samples analysed for stable isotope signature were the same as those in chapter 3 and therefore were collected from the same sites, and identified, prepared, and analysed following the methodology described in chapter 3.

5.2.4 *Competition, community complexity and trophic niche width*

Competition index

An index of competition was derived for each site to investigate the impact of varying competition levels on the trophic niche width of ruffe. The site-specific competition index was calculated based upon the stable isotope signature of organisms within that site. Previous studies have demonstrated that the $\delta^{15}\text{N}$ value of a consumer is enriched by 3.4‰ over that of its diet (Minagawa and Wada 1984; Vander Zanden and Rasmussen 2001). Based upon this, any species in the same community with a $\delta^{15}\text{N}$ value of within $\pm 3.4\text{‰}$ (one trophic level) of the mean ruffe $\delta^{15}\text{N}$ at a particular sampling site was considered to be a potential competitor (as it was feeding within 1 trophic level). The mean $\delta^{15}\text{N}$ and corresponding $\delta^{13}\text{C}$ values for all organisms within a site were plotted on a graph, in a space defined by axes X and Y, and the Euclidean distance was calculated using the following equation:

$$D_{ij} = [(X_j - X_i)^2 + (Y_j - Y_i)^2]^{1/2}$$

Where D_{ij} is the length of the straight line connecting the position of ruffe with a competitor. The reciprocal of this value was taken in order to give more weight to species very close to ruffe in both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. This was carried out for all species with a $\delta^{15}\text{N}$ value within 3.4‰ of ruffe $\delta^{15}\text{N}$ values to produce a ruffe competition index (henceforth the RCI) for each sampling site where:

$\text{RCI} = \sum \text{ of Euclidean distances for all species within } 3.4\text{‰ of ruffe.}$

Community complexity

Two indicators of community complexity were derived for each sampling site (see chapter 3.5). Firstly species richness determined as the simple number of species present was calculated (Table 3.3). The Shannon-Weiner index was also calculated for each site to incorporate the evenness of species recorded (Table 3.3).

Trophic Niche width

In this part of the study, variation in the $\delta^{15}\text{N}$ signature of ruffe from the six sampling sites was used as a proxy for trophic niche width (see chapter 4). Variation in trophic niche of the ruffe population at each of the sampling sites was measured as the population standard deviation. A small standard deviation in a population suggested feeding specialisation leading to a narrow feeding niche width, while a large standard deviation indicated that a more generalist feeding strategy was being adopted, and consequently leading to a broad niche width.

5.2.5 *Determining Fish Condition*

To assess the body condition of ruffe at each sampling site in Loch Lomond, measurements were made of fish lipid content, weight and fork length. Size has been used as a proxy for fitness (Metcalf and Thorpe 1992) and lipid levels have been shown to be highly important in survivorship in fish (Metcalf and Thorpe 1992).

At each of the sampling sites, individual trophic position and ruffe population food chain length were estimated using the method of Vander Zanden and Rasmussen (1999). Within lake differences in $\delta^{15}\text{N}$ values at the base of the food web preclude the use of consumer $\delta^{15}\text{N}$ values as an absolute measure of consumer trophic position. To avoid this difficulty, the trophic position of consumers was estimated by interpreting consumer tissue $\delta^{15}\text{N}$ values relative to the $\delta^{15}\text{N}$ of primary consumers, which were used as indicators of 'baseline isotopic values'. Snail isotopic signatures from each of the six sampling sites were used in this instance to represent baseline values, as snail isotopic signatures have been shown to be a reliable indicator of baseline isotopic signature (Vander Zanden et al. 1999; Post 2002). As in chapter 3.4.7 Baseline correction of stable isotope values was carried out according to Vander Zanden et al (1997).

5.3 Statistical Analysis

Stepwise multiple regression analysis was used to determine which biological and physical factors (Table 5.1) most strongly predict niche width and as such may influence feeding choices. Further analysis was carried out to explore the relationship between community complexity, competition and niche width using simple linear regressions. Finally the effect of variation in niche width on the condition of ruffe at each of the sampling sites was examined using linear regression of niche width upon the lipid deposition, fork length and weight condition factors measured for ruffe at each of the sampling sites.

Regression analysis was used to test for the influence of community characteristics on niche width. The importance of these characteristics may be masked by the use of multivariate statistics due to co-correlation between predictive variables. To overcome this, simple linear regressions were carried out.

5.4 Results

The relationships between niche width, community complexity and competition were tested individually. There are a number of important biological characteristics of communities that in theory would be predicted to have a direct impact upon niche width.

5.4.1 Does competition increase with increasing community complexity?

There was no evidence in this study of a relationship between competition (RCI) and community complexity expressed as species richness ($F_{2,9} = 0.176$, $P = 0.842$; multiple regression using two predictors invertebrate and fish species richness). However, when community complexity was expressed as a Shannon-Weiner value, there was a significant relationship ($F_{2,9} = 5.891$, $P = 0.023$) between competition and community complexity (multiple linear regression using two predictors: invertebrate and fish community Shannon-Weiner indices (Table 5.2)).

Table 5.1 Biotic and abiotic characteristics of sampling sites used in the regression analysis.

Physio chemical characteristics	Exceedince exposure index (EE)	pH	O ₂ % saturation	BOD (mg/l)	Ammonia (mg/l)	Phosphate (mg/l)	Distance from south shore (km)	
Community characteristics	Species richness	Fish Shannon-Weiner	Invertebrate Shannon-Weiner	Food chain length	Fish species richness	Invertebrate species richness	Total competition within 3.4‰ of ruffe δ ¹⁵ N	Average competition within 3.4‰ of ruffe δ ¹⁵ N
Measures of fitness	Average ruffe fork length	Average ruffe weight	Variance in ruffe fork length	Variance in ruffe weight	Average ruffe lipid content	Shannon-Weiner of ruddle stomach contents		

Fifty seven percent of variation in the competition index (RCI) was explained by the fish Shannon-Weiner and invertebrate Shannon-Weiner indices and generated the regression model:

$$\text{Competition} = (-0.0544 * \text{fish Shannon-Weiner}) + (0.220 * \text{invertebrate Shannon-Weiner}) - 0.217$$

Table 5.2 *t*-test of deviation from 0 for intercept and gradients of biological predictors of $\delta^{15}\text{N}$ standard deviation.

Variable	Intercept/gradient	t	p
Intercept	-0.271	-2.215	0.054
Fish Shannon-Weiner	-0.0544	-0.809	0.439
Invertebrate Shannon-Weiner	0.220	3.382	0.008

5.4.2 Is trophic niche width affected by community competition?

The level of competition observed within a community may play a role in determining the niche width of the organisms present within that community, with an increase in interspecific competition leading to a reduction in trophic niche width. However, ruffe collected from the sampling sites in Loch Lomond showed no correlation between interspecific competition and ruffe trophic niche width ($F_{1,10} = 2.252$, $P = 0.164$).

5.4.3 *Does community complexity affect trophic niche width?*

Having demonstrated that a relationship exists between community complexity (expressed as the Shannon-Weiner index) and competition, it may be expected that both of these factors would play an important role in the modulation of the feeding niche width displayed in a single species.

Community complexity has been shown to vary between sampling sites in Loch Lomond (see chapter 3) and theory would suggest that such differences would have the potential to impact upon niche width. Within ruffe populations examined in Loch Lomond there was no significant relationship between community complexity expressed as a species richness score and niche width ($F_{2,9} = 0.160$, $P = 0.854$). This was also the case when community complexity was expressed as a Shannon-Weiner index ($F_{2,9} = 0.195$, $P = 0.826$).

5.4.4 *Do the physical characteristics of a foraging site affect the trophic niche width expressed within a species?*

To attempt to produce a model which predicts trophic niche width, expressed as the standard deviation of $\delta^{15}\text{N}$, a number of physical characteristics (Table 5.1) specific to each site were entered into a forward stepwise multiple regression. Sixty five percent of the variation was explained by the variables BOD (step 1)

and phosphate (step 2) ($F_{2,9} = 8.317$, $P = 0.009$; Table 5.3) and generated the regression model:

$$\delta^{15}\text{N standard deviation} = (-2.409 * \text{BOD}) + (295.9 * \text{PO}_4) + 2.199$$

Table 5.3 *t*-test of deviation from 0 for intercept and gradients of physical predictors of $\delta^{15}\text{N}$ standard deviation.

Variable	Intercept/gradient	t	p
Intercept	2.199	7.902	<0.001
BOD gradient	-2.409	-3.317	0.009
Phosphate gradient	295.893	2.858	0.019

BOD values were positively correlated with niche width and explained thirty three percent of the variation. The second step, phosphate, was negatively correlated with niche width and explained further thirty two percent of the variation. No other variable (see table 5.1 for list) was identified as a significant contributor to the explanation of variance of $\delta^{15}\text{N}$ of ruffe populations at a particular site.

5.4.5 *Do the biological characteristics of a foraging site affect the trophic niche width expressed within a species?*

The biological predictors of niche width (as detailed in Table 5.1) were entered into a forward stepwise multiple regression to determine their role in niche width modulation. No biological characteristic was identified as a significant contributor to the explanation of variance of ruffe population (Table 5.3).

5.4.6 *Are biological or physical characteristics more important in explaining niche width in ruffe?*

Theoretically (see above), both physiochemical and biological factors (related to community structure) have the potential to control niche width. However, in natural systems, neither of these factors work in isolation and it can often be very difficult, if not impossible, to distinguish one from the other. As such it is important to investigate the combined influence of these factors on trophic niche width of ruffe. In this instance, all biotic and abiotic variables listed in Table 5.1 were entered into a forward stepwise multiple regression. Sixty five percent of the variation was explained by the variables BOD (step 1) and phosphate (step 2) ($F_{2,9} = 8.317$, $P = 0.009$;) (Table 5.4) and generated the regression model:

$$\delta^{15}\text{N standard deviation} = (-2.409 * \text{BOD}) + (295.9 * \text{PO}_4) + 2.199$$

No other physical or biological characteristic significantly contributed to the explanation of variation in $\delta^{15}\text{N}$.

Table 5.4 *t*-test of deviation from 0 for intercept and gradients of both physical and biological predictors of $\delta^{15}\text{N}$ standard deviation.

Variable	Intercept/gradient	t	p
Intercept	2.199	7.902	<0.001
BOD gradient	-2.409	-3.317	0.009
Phosphate gradient	295.893	2.858	0.019

5.4.7 Does expressed trophic niche width affect fitness of individuals within a community?

Differences in the standard deviation of $\delta^{15}\text{N}$ (in this instance used as a measure of trophic niche width) were observed for ruffe populations examined at each of the six study sites in Loch Lomond (chapter 3.6.4). Theory would suggest that such changes in trophic niche width would play an important role in dictating individual condition, with a decrease in condition occurring as individuals were forced to reduce their feeding niche width and feed on food of lower nutritional value. Here ruffe body size (fork length and weight) and lipid deposition were used as proxies for body condition. This was analysed using simple linear regression.

There was no significant relationship between trophic niche width and ruffe fork length ($F_{1,10} = 0.012$, $P = 0.916$), ruffe weight ($F_{1,10} = 0.100$, $P = 0.758$), or lipid deposition ($F_{1,10} = 0.182$, $P = 0.679$), although there was a trend towards the reduction of lipid deposition with increasing niche width.

5.5 Discussion

The trophic niche width of an organism is usually described as varying on a continuum from narrow to broad (Begon et al. 1996). The wider the niche, the more generalised the species is considered to be in its prey selection, while the narrower the niche, the more specialised the species is considered to be (Bernays and Minkenberg 1997). Species which have relatively wide niches sacrifice efficiency in the use of a narrow range of resources for the ability to use a wider range of resources (Bernays and Minkenberg 1997). As competitors, organisms with a wide trophic niche are superior to specialists if resources are somewhat undependable (Bernays and Minkenberg 1997). Keast (1979) suggests that this type of feeding plasticity may be particularly advantageous in cold temperate lakes. Organisms with a narrow trophic niche are equipped to exploit a very specific set of resources. As competitors they are superior to generalists if resources are dependable and renewable, as these resources are closely partitioned among specialists to produce low interspecific overlap (Roughgarden 1974).

Within the Loch Lomond system with its narrow, deep, oligotrophic north basin and more nutrient rich, warmer south basin we might expect to see organisms exhibiting a range of trophic niche characteristics. As a result of this, these organisms may provide us with an insight as to the causes and consequences of variation in trophic niche width.

5.5.1 *Niche width, competition and community complexity*

Since Darwin (1859), ecologists have considered interspecific competition, especially competitive exclusion, as the cornerstone of community structure. Lack (1954; 1971), Hutchinson (1959), and Macarthur (1960; 1972), among others, have stressed the role of competition in shaping community organisation, including species distribution, resource allocation and niche segregation.

Since the role of competition in shaping plant communities was discovered, studies have been undertaken to demonstrate the role of interspecific competition in determining community structure, with emphasis on the animal component of communities (Connell 1983; Schoener 1983). Many of these studies suggest, even if they do not prove with certainty, that competition exists between certain species pairs and within guilds of species (Huffaker 1958; Huffaker et al. 1964; Reed 1978).

As community complexity increases, a greater range of food will be available in these more species-rich communities (Martinez 1992; Williams and Martinez 2000). However, where there is evidence of interspecific competition, theory would suggest that this would lead to decreases in niche overlap (Schoener 1974, 1982; Winemiller and Pianka 1990). In this study, there was a significant relationship between the fish and invertebrate Shannon-Weiner scores and competition (see section 5.5.1). However, there was no significant relationship between competition or community complexity and niche width (sections 5.5.2 and 5.5.3).

There are a number of potential explanations for this observation. It may be that sample sizes were not adequate, but due to the high costs associated with stable isotope analysis it was not possible to increase sample numbers. Also, the one system approach adopted in this study greatly reduced the confounding effects of comparing isotopic signature between lochs, but had a potential weakness in that this may have subsequently resulted in lower variation in some community variables.

Generally competition is considered to be asymmetrical, with one species being less affected by competition than the other (Schoener 1983). The nature of ruffe, being a highly opportunistic and plastic species, which is able to maintain feeding at low temperatures, may result in ruffe being less affected by competition than are the native fish of Loch Lomond, and may explain why community complexity and competition was not strongly correlated with niche width in this species.

Classical competition theory assumes that the environment is stable and competition is continuous (Weins 1977), but in reality interspecific competition is probably discontinuous (Weins 1977; Chesson 1986; Weins et al. 1986) because environments are variable and populations are patchily distributed in space and time. It may be the case that in Loch Lomond competition may only occur in certain years in which resources are scarce (Weins 1977).

Furthermore, the relative abundance of a species is not necessarily a measure of its importance to the interactions that characterise the community. An organism's size and activity also play a major role. A large predator, for example, may strongly affect the populations of many other species, even though the predator is not particularly abundant (Jonsson and Ebenman 1998). Furthermore, there is evidence that predation and other forces also play a major role in shaping

ecological communities and it has been suggested that the most important effect of a predator on community structure is to moderate competition among its prey species (Jonsson and Ebenman 1998). This study may have benefited from consideration of factors such as predation that play a role in modification of competition in a community.

5.5.2 *Physical and biological correlates of niche width*

Both the physical and biological characteristics of a site play a role in determining the niche width of an organism (Hutchinson 1957; Carpenter et al. 1987). If the relative importance of these characteristics can be determined, then it may be possible to determine whether communities are tightly or loosely structured entities. If biotic factors are of overriding importance, then communities may be tightly knit entities, however if abiotic forces have the greater influence, then community structure may be loose and ephemeral.

One of the general aims of this chapter was to attempt to find biological and environmental correlates of the observed variation in niche width, which may suggest regulatory mechanisms. A wide range of biological and physical characteristics were tested at each site for concurrent variation in trophic niche width in ruffe and the ability of these factors to predict this variation. Interestingly, none of the biological characteristics tested predicted adequately the trophic niche width variation. However physical characteristics of the site were a good predictor of variation in the trophic niche width of ruffe, suggesting that the community structure at sampling sites in Loch Lomond may be loose and

ephemeral. Specifically Biological Oxygen Demand, an indirect measure of the concentration of biologically degradable material present at each site, predicted 33% of variation in niche width. In a stepwise multiple regression PO_4 predicted a further 32%. Thus 64% of variability in ruffe trophic niche width was predicted by these physiochemical characteristics of the site

5.5.3 Implications of modified niche width on individual fitness

Variations in community complexity, competition and niche width are hypothesised to cause stress and affect body condition. It has been suggested that stress in vertebrates can act on the individual through a physiological feedback involving the endocrine system. The feedback is most closely associated with the functioning of the pituitary and adrenal glands (Christian 1963, 1978; Davis 1978). Stress triggers hyper activation of the hypothalamus-pituitary-adrenocorticular system, which in turn alters the secretion of growth and sex (gonadotrophic) hormones. Profound hormonal changes suppress growth, curtail reproductive functions, and delay sexual activity (Sinclair 1977).

Further these hormonal changes may suppress the immune system and cause the breakdown of white blood cells, increasing an individual's vulnerability to disease (Sinclair 1977). Despite this, there was no significant relationship between body size (fork length or weight) or lipid deposition and niche width, possibly a function of the small sample size at each site. However there was a trend towards a reduction in lipid deposition with increasing niche width which merits further investigation.

Chapter 6. General Discussions

There has been increasing recognition of the importance of food web structure in regulating a wide range of ecologic patterns and processes (Paine 1980; Pimm 1982; Carpenter et al. 1985; Hairston and Hairston 1993; Schindler et al. 1997). However studies of food webs have been limited by poor empirical descriptions of these inherently complex systems and as a result, the conclusions of many of these studies have been limited (Polis 1991) leading to calls for the development of an approach capable of quantifying energy flow and feeding interactions along a single scale (Cohen and Newman 1991; Kenny and Loehle 1991; Martinez 1991; Pimm et al. 1991). The stable isotope approach can provide information on trophic status which is continuous, rather than discrete and also provides an integrated measure of assimilation, making it possible to assess the relative contribution to the food web of resources with distinct isotopic signatures (Lajtha and Michener 1994).

The physical and biological characteristics of Loch Lomond make it an ideal choice of study site for the investigation of energy flows. Within the loch the highland north of the catchment favours the adaptable, phenotypically plastic, generalist species, such as the salmonids, and the conditions in the shallower, more nutrient rich, warmer south in general tend to favour more specialised species, such as the cyprinids (Adams 1994). The diversity of habitat available within littoral sampling sites creates further opportunities for spatial heterogeneity in community structure.

The work presented here was divided into two distinct parts. The first was largely observational, and examined temporal variability in the macrophyte community while attempting to quantify the degree of variability in the invertebrate and fish

communities in Loch Lomond at a local scale. The physical structure of aquatic macrophytes plays a large role in determining habitat heterogeneity in the littoral zone of lakes. Macrophytes can provide food, shelter and refuge for fish, zooplankton and benthic invertebrates and as such play a major role in influencing food web structure (Cyr and Downing 1988; Beklioglu and Moss 1996; Diehl and Kornijów 1998; Jeppesen et al. 1998). Two invasive plant species were identified in Loch Lomond, both from the genus *Elodea*, and the increasing threat of eutrophication (Best and Traill 1994; SEPA 2000a; 2000b) may facilitate the growth and spread of these invading nuisance species adapted to richer nutrient conditions.

In terms of fish and invertebrate community structure, the north and south basins of the loch were similar while the mid basin sites of Ross Point and Salloch Bay produced the most extreme results. For the ruffe, which was ubiquitous across all sites, there was between-site variability in the trophic ecology e.g. diet and variability in its prey items as determined by stomach contents analysis. There was also evidence of seasonal diet variation. Stable isotopes analysis of C and N showed local variation in the stable isotopic signature of ruffe tissues between sampling sites. In addition (as was predicted) there were differences in the stable isotope signature of liver, bone and muscle tissues from the same fish, most likely due to the different turnover rates of these tissues.

The second part of the study focused on a new technique for the investigation of trophic relationships specifically the determination of trophic niche width using stable isotope analysis. This technique was then used to investigate the physical and biological factors governing trophic niche width and the implications for individuals of modified trophic niche width. Within the Loch Lomond system with its narrow, deep, oligotrophic north basin and more nutrient rich, warmer south

basin we might expect to see organisms exhibiting a range of trophic niche characteristics.

Keast (1979) suggests that feeding plasticity may be particularly advantageous in cold temperate lakes. The feeding niche width of an organism is usually described as varying on a continuum from narrow to broad (Begon et al. 1996). Species which have relatively wide niches sacrifice efficiency in the use of a narrow range of resources for the ability to use a wider range of resources (Bernays and Minkenberg 1997). As competitors, organisms with a wide trophic niche are superior to specialists if resources are somewhat undependable (Bernays and Minkenberg 1997). Organisms with a narrow trophic niche are equipped to exploit a very specific set of resources. As competitors they are superior to generalists if resources are dependable and renewable, as these resources are closely partitioned among specialists to produce low interspecific overlap (Roughgarden 1974).

The results presented here show that there is variability in niche width within a single species (*ruffe*) in Loch Lomond, although the pattern of variability is not based on latitudinal variation or a simple differential between basins. Rather this pattern of variation is more of a mosaic with a patchwork of trophic niche width variation in this species (see chapter 3). This observed variation most likely reflects very local variation at a much smaller scale than that of simple basin differences.

As community complexity increases, a greater range of food will be available in these more species-rich communities (Martinez 1992; Williams and Martinez 2000). However, where there is evidence of interspecific competition, theory would suggest that this would lead to decreases in niche overlap (Schoener

1974, 1982; Winemiller and Pianka 1990). In this study, there was a significant relationship between the fish and invertebrate Shannon-Weiner scores and competition (see section 5.5.1). However, there was no significant relationship between competition or community complexity and niche width (sections 5.5.2 and 5.5.3).

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Furthermore, the relative abundance of a species is not necessarily a measure of its importance to the interactions that characterise the community. An organism's size and activity also play a major role. A large predator, for example, may strongly affect the populations of many other species, even though the predator is not particularly abundant (Jonsson and Ebenman 1998). Furthermore, there is evidence that predation and other forces also play a major role in shaping ecological communities and it has been suggested that the most important effect of a predator on community structure is to moderate competition among its prey species (Jonsson and Ebenman 1998). This study may have benefited from consideration of factors such as predation that play a role in modification of competition in a community.

Both the physical and biological characteristics of a site play a role in determining the niche width of an organism (Hutchinson 1957; Carpenter et al. 1987). If the relative importance of these characteristics can be determined, then it may be possible to determine whether communities are tightly or loosely structured entities. If biotic factors are of overriding importance, then communities may be tightly knit entities, however if abiotic forces have the greater influence, then community structure may be loose and ephemeral.

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In summary the main conclusions from this study are:

- Loch Lomond is highly diverse in habitat with 3 main basins but also shows habitat heterogeneity at smaller spatial scales
- Within the littoral zone, diversity is also manifest as variability in the invertebrate and fish community structure
- There is also significant seasonal variability between sites.
- For a single fish species (the ruffe) there is also between-site variability in a number of aspects of its ecology at relatively small spatial scales (e.g. diet, niche width etc).
- There are significant potential strengths in using $\delta^{15}\text{N}$ as a measure of trophic niche width. These are:

- $\delta^{15}\text{N}$ integrates over a more ecologically realistic timescale compared with diet estimated from stomach contents analysis
 - it provides for a single scale over which differing diets can be compared
 - data collection can be fast and non-destructive
 - the integration period can be modified by choosing different tissue types
- Using a simple but realistic model of likely standard deviations in $\delta^{15}\text{N}$ (from the literature), estimates of the statistical precision of limits of change using $\delta^{15}\text{N}$ as an index of trophic niche width showed that:
 - foraging specialist populations (with a low standard deviation in $\delta^{15}\text{N}$) would need a smaller numerical change in $\delta^{15}\text{N}$ standard deviation than would a generalist population.
 - the diet shift required to detect change in extreme but realistic generalist and specialist populations are biologically realistic.
- There is empirical evidence from this study that standard deviation in $\delta^{15}\text{N}$ for ruffe from Loch Lomond does adequately predict real trophic niche width (based on stomach contents analysis).
- Here there was no evidence of a hypothesised relationship between community competition and trophic niche width for Loch Lomond ruffe

- There was no evidence for a relationship between maximum trophic chain length and trophic niche width.
- Nor for an effect of community complexity on ruffe trophic niche width.
- Physiochemical characteristics of sites significantly predicted variation in ruffe trophic niche width but evidently no elements of community structure had an effect on trophic niche width of ruffe in this study.
- The best predictor of trophic niche width in ruffe was biological oxygen demand, with water phosphate concentration also contributing to the variation in ruffe trophic niche width.
- Variability in trophic niche width did not seem to consequences for two measures of fitness in ruffe (lipid deposition levels, growth or overall size).

Appendices

Appendix 1 Temporal and Distributional Variation in Submerged Macrophyte Communities of Loch Lomond, Scotland

Variation Temporel et Spatial des Communautés de Macrophytes Submergées de Loch Lomond, Écosse

Temporal and Distributional Variation in Submerged Macrophyte Communities of Loch Lomond, Scotland

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Résumé: Les valeurs de la biomasse de macrophytes submergées ont été évalués de mai à octobre 2001 aux sites dans les trois bassins de Loch Lomond. Les données ont été utilisées pour identifier les communautés présentes, les espèces dominantes, et les variations des saisons dans et entre les bassins. Une comparaison a été fait avec les données d'une investigation précédente, en 1990, pour qualifier les changements de longue terme. L'introduction des espèces pas-indigène, et les changements chimiques de l'eau de la lac, sont menaces aux communautés de macrophytes submergées de Loch Lomond.

Mots clés: Loch Lomond, eutrophication, plantes invasives

Abstract

Submerged macrophyte biomass values were assessed monthly from May to October 2001 from sites within the three basins of Loch Lomond. The data were used to identify communities present, dominant species and seasonal variation within and between basins. A comparison is made with data from a similar survey carried out in 1990 and used to assess longer-term changes. The introduction of non-native species and changing nutrient levels are identified as threats to the macrophyte communities of Loch Lomond.

Key words

Loch Lomond, eutrophication, invasive species

Introduction

Loch Lomond is Britain's largest area of fresh water (70.27km²). It experiences heavy recreational use, and is also used for hydroelectric power and potable water supply. Loch Lomond supports a wide range of freshwater plant communities located along gradients of environmental conditions. Loch Lomond and its surrounding area will form Scotland's first National Park from 2002, with new legal restrictions likely on planning, water quality protection and recreational use. In this context there is a need for hard information on the natural resource base of the loch, and how it is changing in response to new pressures on the ecosystem. The data gathered were used to identify communities present, seasonal changes in macrophyte biomass and also to identify differences in the level of macrophyte production between basins. Eutrophication (Best & Traill 1994) is an increasing threat to these aquatic plants and may facilitate the growth and spread of invading species adapted to richer nutrient conditions. These may outcompete and exclude native submerged species adapted for growth in oligotrophic-mesotrophic conditions. In order to assess longer-term trends of change within the loch system, a comparison is made with similar survey data from Loch Lomond collected in 1990 (Murphy et al. 1994) which identified three separate euhydrophyte communities in the loch.

Methods

Monthly surveys of submerged macrophyte biomass were carried out in the three basins (South, Mid and North basins) of Loch Lomond during May to October 2001. Four sites with varying intensities of wind exposure were selected from each basin. From each of these sites, three Ekman grab samples (area 0.155m x 0.155m) were taken. All plant material within the sample was collected, washed, identified to species and dried at 60°C before weighing. TWINSpan (Hill 1979) analysis of combined 1990 and 2001 data is used to identify trends of change in the macrophyte communities of Loch Lomond over the 11 year period.

Results

The results suggest that, as in 1990 (Murphy *et al* 1994), *Littorella uniflora* is the dominant species in Loch Lomond, followed by *Isoetes lacustris* and *Myriophyllum alterniflorum* (Figure 1).

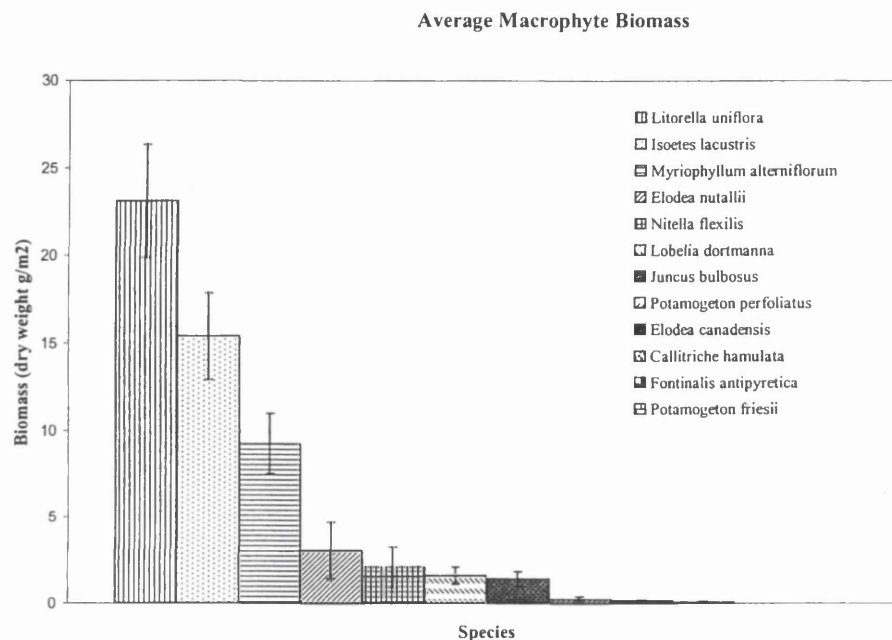


Figure 1. Average macrophyte biomass (g/m^2 dry weight \pm standard error) recorded for Loch Lomond over the period May to October 2001.

During the 1990 survey, Murphy *et al.* (1994) recorded the spread of *Elodea canadensis*, thought to have invaded Loch Lomond between the time of Idle's survey in 1967 (summarised by Bailey-Watts 1981) and a Glasgow University field course in 1988 when it was one of the common species recorded. Since 1990, a second invasive species has colonised the loch. *Elodea nuttallii* is now present throughout the length of Loch Lomond and in the relatively short space of time since its appearance, has spread rapidly.

Spence (1964) calculated that up to 20% of the total surface area of Loch Lomond is suitable for plant growth. Of this, he estimated that only 1% is actually colonised by plants. The majority of this area lies within the South basin. Data collected in 2001 (Figure 2) suggest that biomass values are lower in the mid basin of Loch Lomond, possibly due to higher shoreline exposure in this area. The South and North basin sampling sites show similar levels of macrophyte production although areas suitable for macrophyte colonisation are much less in the North basin than in the South basin.

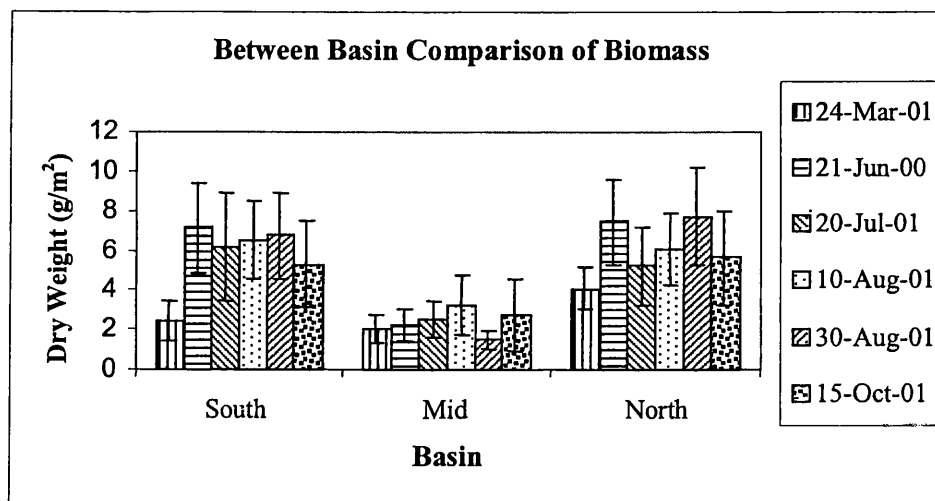


Figure 2. Between basin comparison of average monthly macrophyte biomass ($\text{g/m}^2 \pm$ standard error)

Table 1. Two-way table of sites x species produced by TWINSpan for the combined 1990 and 2001 Loch Lomond vegetation data sets. Groups A-C are shown with indicator species underlined.

Groups:	B	C	A
Sites:	11	1222 11211111	
	02178395269013456217834		
4 <u>Elod cana</u>	-1-----111111-111---11		0000
12 Pers amph	-----1-----		0001
13 Pota frie	-----1-----		0001
14 <u>Pota perf</u>	1-----1-11---1-1--1--		0001
20 Ranu pelt	-----1-----		0001
6 Isoe lacu	111111111111-111111---11		0010
9 Litt unif	111111111111111111-1111		0010
11 Myri alte	11-1111111111111111--11		0010
21 Nite flex	-1--1111-----11111111-		0010
23 <u>Elod nutt</u>	111--11111-----		0011
10 <u>Lobe dort</u>	11111-----11		01
17 Font anti	-----1-----1		100
1 Apiu inun	-----1-----		1010
3 Care spp	-----1-----		1010
5 Hydr vulg	-----1-----		1010
7 Junc acut	-----1-----		1010
15 Spar angu	-----1-----		1010
16 Eurh prae	-----1-----		1010
18 Spha cusp	-----1-----		1010
19 Spha subs	-----1-----		1010
22 Spha sp	-----1-----		1010
24 <u>Utri sp</u>	-----11		1010
25 Leaf live	-----1-----		1010
8 Junc bulb	-----1--11		1011
2 Call hamu	11-----11		11
	0000000000000000000011		
	0000000111111111111111		

Note: [-] = absent from site; [1] = present in the site; numbers in **bold** represent 2001 sampling sites (site codes: read downwards). Sample codes: South basin 1990:15 16 17 18 19 20 & 21; 2001:1 2 3 & 4; Mid basin 1990:22 & 23; 2001:5 6 7 & 8; North basin 1990:13 & 14; 2001:9 10 11 & 12. Key to species: Apiu inun: *Apium inundatum*; Call hamu: *Callitriche hamulata*; Elod cana: *Elodea canadensis*; Elod nutt: *Elodea nuttallii*; Eurh prae: *Eurynchium praelongum*; Font anti: *Fontinalis antipyretica*; Hydr vulg: *Hydrocotyle vulgaris*; Isoe lacu: *Isoetes lacustris*; Junc acut: *Juncus acutiflorus*; Junc bulb: *Juncus bulbosus*; Leaf live: Leafy liverwort; Litt unif: *Littorella uniflora*; Lobe dort: *Lobelia dortmanna*; Myri alte: *Muriophyllum alterniflorum*; Nite flex: *Nitella flexilis*; Pers amph: *Persicaria amphibia*; Pota frie: *Potamogeton friesii*; Pota perf: *Potamogeton perfoliatus*; Ranu pelt: *Ranunculus peltatus*; Spar angu: *Sphagnum angustifolium*; Spha sp: *Sphagnum* sp.; Spha cusp: *Sphagnum cuspidatum*; Spha subs: *Sphagnum subsecundum*; Utri sp: *Utricularia* sp.

Comparison of 1990 and 2001 data using TWINSpan analysis identified, at level 2 of the divisive classification, three main community types, labelled A-C in Table 1. Group A comprises the two sites with the highest diversity of species, both of which are 1990 sites. The indicator species for this group is *Utricularia* sp. Group B is made up entirely of 2001 sampling sites. The indicator species for this group are *Elodea nuttallii* and *Lobelia dortmanna*. The remaining sites make up group C, with *Elodea canadensis* and *Potamogeton perfoliatus* as indicators. That *E. nuttallii* and *E. canadensis* serve as indicator species for different groups is significant since it was initially thought that *E. nuttallii* would outcompete and replace *E. canadensis* throughout Loch Lomond: this has not yet happened.

Conclusion

The introduction of non-native aquatic macrophyte species, coupled with changing nutrient levels, pose a very real threat to the euhydrophyte communities of Loch Lomond. Significant changes have already taken place. Although it is almost impossible to prevent the introduction of non native species to the loch, the question of eutrophication is one which must be addressed in order to prevent these species outcompeting and excluding native species adapted for growth in this oligotrophic-mesotrophic waterbody.

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Appendix 2 Invertebrate stable isotope values

	Inverhoulin		Rubha Mor		Ross Point		Sallochay Bay		Inchlonaig		Ross Priory	
	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
Asellidae	6.16	-19.14	2.88	-16.25	4.16	-22.35	4.36	-20.31	4.08	-14.46	4.98	-17.08
Baetidae	3.67	-23.87										
Caenidae	7.74	-17.88	-1.92	-15.68								
Sphaeriidae					4.81	-23.7	4.51	-23.76			5.87	-24.5
Physidae	4.1	-17.91	3.64	-15.53	4.34	-26.89			3.73	-14.11	5.36	-14.4
Lymnaeidae	4.13	-17.06	2.25	-15.12	3.01	-14.52	3.83	-14.26	4.06	-13.91	3.92	-14.81
Spire shell											6.02	-19.6
Ramshorn	3.36	-15.13	3.75	-13.56	4.34	-20.27	5.55	-16.91	3.36	-15.13	5.62	-19.13
Coleoptera larvae	6.74	-20.65	2.29	-21.52	2.03	-23.48						
Gammaridae	6.79	-17.75	6.87	-21.02	7.43	-26.45	4.72	-18.2	7.7	-18.29	7.33	-19.3
Ceratopogonidae					5.75	-16.14	5.93	-18.76				
Chironomidae	7.37	-21.23	2.35	-18.87	7.01	-22.19	5.16	-18.17	4.36	-15.17	6.32	-18.8
Oligochaeta	7.19	-23.05	3.16	-18.95	9.36	-21.71	4.86	-20.72	4.15	-16.91	8.71	-20.04
Tricladida	11.27	-22.53	4.96	-23.02	6.11	-19.73	7.09	-21.17	6.07	-17.32	8.32	-18.96
Hirudinea	6.83	-21.95			7.54	-19.95	4.89	-13	5.11	-13.86	8.32	-18.96
Eggs	5.49	-28.04	2.9	-28.86	4.61	-26.38	2.56	-25.04	3.59	-25.96	4.38	-24.66
	Inverhoulin		Rubha Mor		Ross Point		Sallochay Bay		Inchlonaig		Ross Priory	

	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
Trichoptera cased	3.35	-18.47	4.99	-21.86	3.49	-24.5	3.94	-23.46	6.15	-20.58	5.73	-21.58
Trichoptera uncased			2.96	-15.46	7.73	-19.6	7.73	-19.6	6.96	-14.95	7.96	-21.36
Hydracarina	9.31	-18.64	3.97	-20.82	7.37	-22.42	6.83	-20.59	8.21	-23.05	9.05	-27.53

Invertebrate stable isotope values from six sampling sites in Loch Lomond. Results are non baseline corrected and lipid was not extracted from samples prior to analysis.

Determining trophic niche width: a novel approach using stable isotope analysis

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Summary

1. Although conceptually robust, it has proven difficult to find practical measures of niche width that are simple to obtain, yet provide an adequate descriptor of the ecological position of the population examined.
2. Trophic niche has proven more tractable than other niche dimensions. However, indices used as a proxy for trophic niche width often suffer from the following difficulties. Such indices rarely lie along a single scale making comparisons between populations or species difficult; have difficulty in combining dietary prey diversity and evenness in an ecologically meaningful way; and fail to integrate diet over ecological time-scales thus usually only comprise single snapshots of niche width.
3. We propose an alternative novel method for the comparison of trophic niche width: the use of variance of tissue stable isotope ratios, especially those of nitrogen and carbon.
4. This approach is a potentially powerful method of measuring trophic niche width, particularly if combined with conventional approaches, because: it provides a single measure on a continuous axis that is common to all species; it integrates information on only assimilated prey over time; the integration period changes with choice of tissue sampled; and data production is theoretically fast and testing among populations simple.
5. Empirical studies are now required to test the benefits of using isotopic variance as a measure of niche width, and in doing so help refine this approach.

Key-words: carbon isotope, diet, generalist, nitrogen isotope, specialist.

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Introduction

Hutchinson's (1957) conceptualization of niche as an n -dimensional hypervolume was a crucial foundation upon which ecologists have tried to understand the development of community structure. Occupied niche space implies resource use, and understanding factors that lead to change in niche parameters is central to understanding the evolutionary process. For example ecological character displacement is regarded as a

key driving force by evolutionary ecologists (Losos 2000) and cladogenesis can be viewed as an emergent property of competition for resources (Bridle & Jiggins 2000).

Niche parameters can respond very rapidly to changes in intraspecific and interspecific competition as well as prey abundance. For example, competition for niche space is relaxed on islands as a consequence of species impoverishment, thus insular forms typically show an expanded niche width relative to their mainland counterparts (MacArthur, Diamond & Karr 1972). Differences in niche width are conventionally demonstrated using proxies such as bill size (Grant 1965; Gosler & Carruthers 1994), body size (Grant 1968; Clegg & Owens 2002), feeding ecology or prey preferences

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(Carrascal, Moreno & Valido 1994; McDonald 2002) and habitat choice (Diamond 1970; MacArthur *et al.* 1972; Blondel, Chessel & Frochot 1988).

Niche width is usually expressed by calculating the heterogeneity within a set of ecological measurements, often borrowing indices derived as measures of evenness and richness (Shannon & Weaver 1949; Simpson 1949; Margalef 1958). Trophic niche width, often assessed using dietary diversity, is the most tractable and frequently studied component of niche space. However, there are practical problems associated with quantifying trophic niche width using conventional dietary analysis.

1. It is difficult to measure accurately the relative abundance of differing dietary prey items, and over- or underestimates are possible. For example, pellet-content analyses overestimate the proportion of birds in the diet of great skuas *Catharacta skua* (Brunnich) (Votier *et al.* 2001).
2. Temporal integration of dietary information is often difficult to quantify, such that many dietary studies are 'snapshots' of dietary prey at a point in time.
3. Conventional dietary analysis techniques, in most instances, are unable to take account of variation in prey assimilation rates.

In addition to these observational biases, conventional dietary analysis is often intrusive and can be cumbersome and labour-intensive. For example, a crucial question to ask of a population that appears to show a large dietary niche width is whether it is composed of generalist individuals all taking a wide range of food types (Type A generalization), or individuals each specializing on a different but narrow range of food types (Type B generalization) (Van Valen 1965; Grant *et al.* 1976). Distinguishing the form of population generalization is important for constructing evolutionary hypotheses (e.g. Clegg & Owens 2002), but discriminating between the alternatives using conventional approaches requires laborious sampling of individuals over extended time periods followed by integration of the information, which is often difficult to achieve.

Criteria defining a useful and robust measure of dietary niche width should (i) allow direct comparison amongst individuals, populations and species through the arrangement of samples along a single diversity scale; (ii) combine information on richness and evenness of dietary composition; and (3) allow temporal integration of dietary information over different time-scales, preferably from a single sampling event.

Currently we have no robust measure of niche width that satisfies all of these basic requirements for practical application. Of the criteria least frequently met by current techniques, is the ability to compare between populations and species on a single scale. Here we propose a new method that meets all of the basic requirements listed, is theoretically strong, and is simple to apply; namely the use of variance in stable isotope ratios of consumer tissues (see also Matthews & Mazumder 2004).

Conventional applications of stable isotope analysis to ecology

Over the past 15 years, stable isotope ratios of nitrogen and carbon have been used increasingly by animal ecologists to elucidate patterns in food webs. Their utility lies in the fact that stable isotope ratios in the proteins of consumers reflect those of the proteins in their diet in a predictable manner (Hobson & Clark 1992a; Hobson 1999a). Conventionally expressed as $\delta^{15}\text{N}$ (‰), the ratio of ^{15}N to ^{14}N generally exhibits a stepwise enrichment (increase in the value of $\delta^{15}\text{N}$) at each trophic level and consequently the $\delta^{15}\text{N}$ values in the tissues of consumers tend to be between 2–5‰ and 5‰ greater than those of their diets (e.g. DeNiro & Epstein 1981; Hobson & Clark 1992b; Bearhop *et al.* 2002). The ratio of ^{13}C to ^{12}C ($\delta^{13}\text{C}$) also increases with trophic level, but to a much lesser degree than $\delta^{15}\text{N}$, in the order of 1‰ (e.g. DeNiro & Epstein 1978).

Carbon and nitrogen stable isotope ratios at the base of food webs may also vary spatially, and this is reflected in spatial variability in isotopic composition among food webs. Such spatial variability can be on a grand scale – for example the difference in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of basal marine food webs resources from that of a terrestrial food web is reflected throughout all of the species within each web (Hobson 1999a) – or on a smaller scale – geographical differences in baseline $\delta^{15}\text{N}$ signatures may occur within the same category of ecosystem (Hobson 1999b; Vander Zanden & Rasmussen 2001). Such differences are often to the observer's advantage. For example, spatial variability in $\delta^{13}\text{C}$ can reveal the relative importance of other carbon pools to a consumer, discriminating between inshore and offshore feeding at a variety of spatial scales, from the open sea (Hobson, Piatt & Pitocechelli 1994) to relatively small freshwater lakes (France 1995), or by helping distinguish animals feeding in moist primary forests from those feeding in drier second growth scrub (Marra, Hobson & Holmes 1998).

The carbon and nitrogen isotopic composition of consumer tissues are thus a function of: $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of each prey species; the relative proportions of each prey species assimilated; the isotopic fractionation associated with converting prey tissue into consumer tissue; and in certain instances, foraging location. Moreover, the stable isotope signatures of tissues generally reflect the diet over the period during which the tissue was synthesized (Hobson & Clark 1992a; Bearhop *et al.* 2002), such that tissues with different turnover rates will integrate dietary information over different temporal periods. For example, blood is a short-term integrator whereas bone integrates the dietary nitrogen over a much longer time-scale (Hobson & Clark 1992a; Haramis *et al.* 2001; Bearhop *et al.* 2002; Pearson *et al.* 2003). Finally, tissues that are metabolically inert after formation, such as hair, feathers, baleen or claws, will preserve this record indefinitely (Schell, Saupe & Haubenstock 1989; Hobson 1999a; Bearhop *et al.* 2003).

Combined, such qualities render stable isotope analysis a powerful tool to study diet. However, to date relatively few studies have given thought to the variation associated with the mean isotopic signature (e.g. Genner *et al.* 1999; Bearhop *et al.* 1999), which when combined with conventional assessment of diet, we propose has the potential to be a powerful integrative measure of foraging niche width.

Stable isotope variance as a measure of niche width

For this approach to provide a useful measure of niche width we make the following theoretical assumptions.

1. Prey species must differ isotopically. This can be assessed by isotopic characterization of potential prey items. If variation does not exist then this assumption would be invalid, and further consideration of niche width (through isotopic variance of the consumer) would be futile.
2. The isotope signatures at the food-web base, and the diets of prey species remain relatively invariant over time. Several studies have shown that baseline isotope signatures can change over time as a consequence of primary production shifts or nutrient inputs, and dietary preferences of prey may also change (Yoshioka, Wada & Hayashi 1994). In practice, if the isotopic signature of the (combined) prey exhibits temporal variation, as long as this variance is less than the variance resulting from a consumer dietary shift (revealed by sampling of prey items), stable isotope signature variance should remain a robust measure of trophic niche width.
3. The tissue analysed reflects the period over which the niche width is expressed. In a population of generalists (particularly Type A generalists) variability in diet amongst individuals will tend to exist at only shorter temporal scales, and this variation is likely to become lost through averaging of the stable isotope signature over longer periods. In this case, tissues with integration times slightly shorter than the period of niche width assessment will likely provide the best indicators of niche width. However, where a whole population shifts diet synchronously for comparison with a population where individuals shift asynchronously, serial sampling of tissues integrating relatively short-term information would be required. In keeping with more traditional approaches to trophic niche-width estimation, the detail of the question being asked will determine the most appropriate choice of tissue.

Where these assumptions are met, we propose the following will influence the isotopic variance exhibited by a consumer population, or an individual serially sampled over time:

1. the range of prey species consumed;
2. the evenness (in its ecological sense) of prey components in the diet over time;
3. the range of trophic levels from which prey is drawn;
4. foraging location;
5. variability in individual physiology; and
6. variability in diet-tissue fractionation.

Numbers 1–4 have been used previously as indicators of foraging niche width; numbers 5 and 6 should, in most cases, result in small variations in stable isotope variance and thus add only a small amount of noise to variance estimates. Here we consider the effect each control will exert in more detail and derive specific predictions relating to the use of stable isotope analyses as a measure of foraging niche width. At this stage in isotope ecology studies, due to larger trophic differences and proportionally smaller measurement precision, variance in $\delta^{15}\text{N}$ is the most powerful parameter to consider, thus much of the following discussion will focus on this, although with respect to geographical foraging area $\delta^{13}\text{C}$ may offer considerable utility.

(1) THE RANGE OF PREY SPECIES CONSUMED

Prediction 1: in general, populations that consume a wide range of prey species will exhibit wider variation in their tissue isotopic signatures than those consuming a narrow range of prey items. For example, a population of shags *Phalacrocorax aristotelis* (L.) that fed exclusively on a single prey type at a single foraging site had a smaller variance in $\delta^{15}\text{N}$ (feather) (0.33‰) than feathers of cormorants *Phalacrocorax carbo* (L.) which had been feeding on multiple prey types at multiple sites (4.04‰) (Bearhop *et al.* 1999).

(2) THE EVENNESS OF PREY COMPONENTS IN THE DIET OVER TIME

Prediction 2: populations where individuals consume widely differing proportions of each of their prey items over time will tend to show less variation in tissue stable isotope ratios than will those consuming a constant proportion of each prey type. This is demonstrated in Fig. 1. Further, asynchronous population diet switching would lead to large isotopic variability, synchronous population variation would lead to small isotopic variability. Detecting whether variation was asynchronous

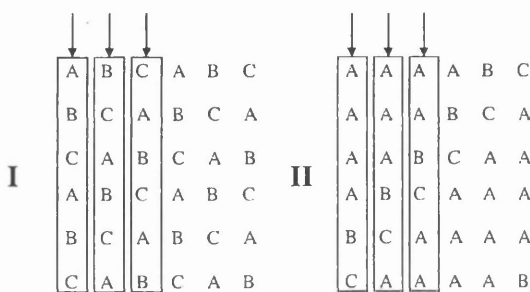
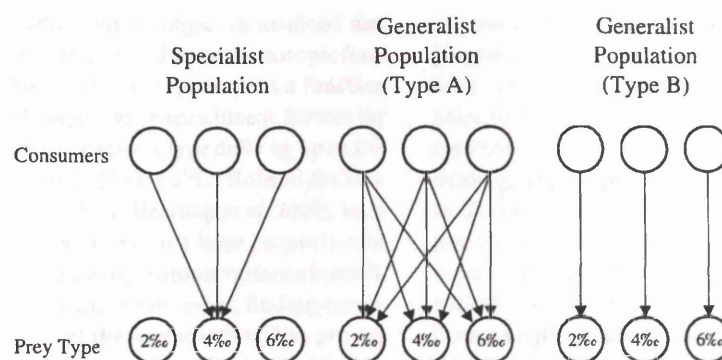


Fig. 1. The matrices represent two populations, one with high evenness in the diet (I) the other with low evenness in the diet (II). Each row represents the diet of a different individual over time and each letter a different type of prey item. The arrows indicate a sampling event and the boxes show the population sampled at each sampling point. Because the prey preferences of the consumer populations do not fluctuate synchronously, sampling a population with high dietary evenness will tend to yield higher variances than one with low evenness.



If we assume that diet/tissue fractionation is constant (4‰), prey isotope ratios remain constant over time and that Type A individuals consume all prey types in equal amounts then:

(A) Sampling a tissue that integrated dietary information over long temporal scales would likely give consumer population values (mean \pm s^2) of

Specialist	Generalist (Type A)	Generalist (Type B)
8‰ \pm 0	8‰ \pm 0	8‰ \pm 4

(B) Sampling a tissue that integrated dietary information over short temporal scales (with a large sample size) would likely give consumer population values (mean \pm s^2) of

Specialist	Generalist (Type A)	Generalist (Type B)
8‰ \pm 0	8‰ \pm 4	8‰ \pm 4

(C) Assuming that the tissue being sampled integrates dietary information over a shorter period than the diet varies over, serial sampling the same tissue (integrating very short-term dietary information, such as blood plasma samples, or short sections from feathers, hair or possibly whiskers) from the same individual over time would likely give individual values (mean \pm s^2) of

Specialist	Generalist (Type A)	Generalist (Type B)
8‰ \pm 0	8‰ \pm 4	6, 8 or 10‰ \pm 0

Fig. 2. Sampling regimes that could enable the use of stable isotope variance in animal tissues to discriminate between Type A and Type B generalism. For clarity, the examples represent idealized predator–prey systems, where dietary specializations represent extremes of the specialist/generalist and type I/type II continua and the problems of estimating population-level variances are ignored.

/synchronous between two populations would be possible through a sampling regime similar to Fig. 2 (part (c)).

(3) THE RANGE OF TROPHIC LEVELS FROM WHICH PREY IS DRAWN

Prediction 3: populations where individuals consume prey over a broad spectrum of trophic levels will tend to show more isotopic variance than those which feed on the same number of prey species, but all drawn from the same trophic level.

(4) GEOGRAPHIC FORAGING AREA

Prediction 4: since spatial variation at the food-web base is reflected throughout the food web, populations where individuals forage in a range of geographical areas are likely to show more variation in the stable isotope signatures of their tissues than those from sedentary populations.

(5) VARIABILITY IN INDIVIDUAL PHYSIOLOGY

Prediction 5: physiological differences among individuals within the population (or within the same individual

over time) will cause some variance in tissue-isotope signatures. A first consideration should be variability in nutritional condition. For example, the tissues of individuals in poor nutritional condition had elevated $\delta^{15}\text{N}$ compared to those of individuals in better condition (Hobson, Alisauskas & Clark 1993). Variability in metabolic rates may also lead to inter-individual variation in tissue isotope signatures (observed during studies of captive individuals; Hobson & Clark 1992a; Bearhop *et al.* 2002). However the magnitude of this effect on population or serially sampled individual variance is likely to be small and would manifest in noise, rather than forcing error between populations or individuals. Nevertheless, despite recent advances, our understanding of how variability in physiology influences tissue stable isotope signatures is still limited, and more work is required in this area.

(6) VARIABILITY IN DIET-TISSUE FRACTIONATION

Prediction 6: diet-tissue isotopic fractionation may vary with the type of food being consumed or through differential mobilization of stored resources (Adams &

Sterner 2000). Captive birds subject to artificial diet switches exhibited variation in diet-tissue isotopic fractionation (Bearhop *et al.* 2002), perhaps as a function of diet quality. However, since enrichment factors for different diets to the same tissue type differ by up to 2‰ for $\delta^{15}\text{N}$ and by just over 1‰ for $\delta^{13}\text{C}$ (Hobson & Clark 1992b; Haramis *et al.* 2001; Bearhop *et al.* 2002), such variability may only account for a large proportion of the variance when the dietary isotopic variance is small.

Despite a thorough literature review, finding empirical studies to support these predictions has proved extremely difficult since data have not been collected with these hypotheses in mind (to our knowledge). For example there are few studies of wild populations where serial sampling of the same tissue type (or individual) has been undertaken contemporaneous with monitoring isotopic composition of the diet, or insufficient individuals from comparable populations have been measured to allow the appropriate statistical analyses. However, with relatively simple sampling protocols, and the appropriate experimental design, there is the potential to address a number of questions with respect to niche width. For example, the question posed in the introduction regarding the manner in which niche width is expressed (i.e. Type A or Type B generalists) could be investigated in the manner described in Fig. 2.

DISCRIMINATING BETWEEN POPULATION AND INDIVIDUAL GENERALISM

Using conventional methods to address this problem has required labour intensive field observations and often populations of identifiable individuals. However, either by serial sampling or utilizing the differential rate of tissue turnover, stable isotope analysis offers a powerful approach to estimate the relative prevalence of population, and individual, generalism. Because different animal tissues integrate dietary signatures over different temporal scales (Hobson & Clark 1992a; Bearhop *et al.* 2002), in a population of generalists we predict the variance among tissues that integrate diet over short temporal scales (shorter than period of trophic variation) to be larger than the variance for tissues that integrate diet over longer temporal scales (that cover the period of trophic variation). Thus for example, tissues that integrate over days and weeks, such as blood plasma, blood cells or individual feathers (Hobson & Clark 1992b; Hilderbrand *et al.* 1996; Bearhop *et al.* 2002), are much more likely to discriminate dietary generalism than tissues which integrate variation over much longer time-scales, such as bone, groups of feathers, fish otoliths or scales (Hobson & Clark 1992a; Begg & Weidman 2001). It follows that if we have a population of specialists we would predict little or no change in variance between long- and short-term integrators (Fig. 2, parts (a) and (b)).

If individuals within the population were identifiable, serial sampling from the same individuals would also distinguish Type A and Type B generalists. Serial

sampling could comprise multiple blood samples, sampling sections of feathers grown at different times in the moult cycle or sampling multiple subsections of long hairs such as vibrissae. We would expect the variation measured sequentially within individuals from population of Type A generalists to be approximately equal to the variation found in sample representative of the population, whilst for Type B generalism, we would expect variance derived from sequentially measured individuals to be low compared with the variance derived from a single sample of the population at any one time (Fig. 2, part (c)). This latter approach, although potentially increasing animal stress in the case of blood due to multiple re-captures, would in general be more desirable than the multiple tissue approach, which may require the sacrifice of animals.

Closing remarks

We conclude that using variance in stable isotope analysis, particularly of $\delta^{15}\text{N}$, may offer a significant addition to the range of techniques for estimating trophic niche width in animals and comparisons can be undertaken using a simple variance ratio test (*F*-test). The technique, potentially at its most powerful when combined with conventional approaches, would be best applied in closed systems, or where nutrient inputs or changes in production could be easily quantified, such as freshwater lakes or islands. Under certain circumstances marine systems, which tend to be more isotopically homogeneous (over moderate spatial and temporal scales), may be suitable. Potential confounding effects of physiology should also be considered. While our understanding of physiological effects upon tissue stable isotope signatures has increased considerably in recent years due to an increase in the number of controlled dietary studies (Hobson & Clark 1992a, 1992b; Hilderbrand *et al.* 1996; Haramis *et al.* 2001; Bearhop *et al.* 2002; Pearson *et al.* 2003), more work of this nature is required. We suggest that the technique could provide valuable insights into the processes underlying insular evolution and the impacts of alien introductions upon the communities they invade. The challenge now lies with the ecological community to evaluate fully the usefulness of this approach through the design and execution of empirical studies that use isotopic variance as a measure of niche width.

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